21st International Symposium on Chromaffin Cell Biology

www.ISCCB.org

Hotel Hafen Hamburg, Germany 7-12 July 2022
Seewartenstraße 9, 20459 Hamburg, Germany

Organizing Committee:

Manfred Lindau, University of Miami, FL
Elizabeth (Liz) Seward, University of Sheffield, UK
Ricardo Borges, Universidad de La Laguna, Tenerife, Spain
Ute Becherer, Saarland University in Homburg, Germany

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We thank our sponsors!

Thursday 7th

16:30 **Registration. Foyer Elbkuppel**

17:00-19:00 **Opening Reception. Foyer Elbkuppel, Hotel Hafen Hamburg**

19:00-19:45 **Introductory Lecture. Foyer Elbkuppel**
Antonio Garcia (Universidad Autónoma de Madrid, Spain)
Forty years of chromaffin cell biology around the world

21:00-22:30 **Guided visits: Hamburg's St. Pauli and Harbour Walking Tours**
Experience the glittering entertainment mile with theaters, clubs, pubs, live music and table dance bars around the world famous Reeperbahn. Our local guides show you how new clubs und theaters changed the "Spielbudenplatz", where the night-owls go out and what is behind the "no admission" for women in the "Herbertstrasse". Hear stories and anecdotes about life in the quarter, the red-light district and about the times where the Beatles started their career in St. Pauli. The walk leads also to "Große Freiheit", the "Hans Albers square", the "Ritze" and interesting side streets of the red-light district. The St. Pauli quarter is a dockland area where you hear the sounds of the harbor. Many dock workers used to live here and it is here where the vessels landed and the sailors flocked to St. Pauli to have some fun. The guides will show you in what rip-off taverns you can lose your money fast so you want to avoid those. They will show where street prostitution is legal and, importantly, that St. Pauli is much more than an amusement quarter, it is a place where many people live who love it and call it their home. The walking tour includes the Millerntorplatz, the Dancing Towers, Reeperbahn, Spielbudenplatz, Hans-Albers-Platz, Große Freiheit and the streets Hamburger Berg, Seilerstraße und Schmuckstraße.

Friday 8th ISCCB-21

08:00-17:00 **ISCCB office opens. Foyer Elbkuppel**

08:30 **Coffee, Tea and Snacks. Foyer Elbkuppel**

09:00-10:30 **Early morning session: SNAREs & Associates I. Elbkuppel**

Chair: Nikhil Gandasi and Ira Milosevic

09:00 Reinhard Jahn (MPI for Multidisciplinary Sciences, Göttingen, Germany)
Intermediate steps in SNARE-mediated membrane fusion

09:30 Christian Rosenmund (Charité, Berlin, Germany)
Syntaxin-1A modulates vesicle fusion in mammalian neurons via juxtamembrane domain dependent palmitoylation of its transmembrane domain

10:00 Jose Rizo (UT Southwestern Medical Center at Dallas TX, USA)
Understanding neurotransmitter release in atomic detail

10:30 **Biochemical Society Lecture, Elbkuppel**

Nobel Laureate Dr. Erwin Neher (MPI for Multidisciplinary Sciences, Göttingen, Germany)
Dynamic priming in both, chromaffin cells and synaptic terminals

This lecture is sponsored by the Biochemical Society

11:00 **Morning Coffee Break & Posters view. Foyer Elbkuppel**

11:30-12:30 **First Plenary Talk. Elbkuppel**

Chair: Manfred Lindau
Axel Brunger (Stanford University, USA)
Molecular mechanisms of calcium-triggered exocytosis

12:30-13:30 **Late morning session: SNAREs & Associates II. Elbkuppel**

Chair:

12:30 Yongli Zhang (Yale University, USA)
Energetics and kinetics of chaperoned SNARE assembly revealed by optical tweezers

13:00 Volker Kiessling (University of Virginia, Charlottesville VA, USA)
Fusion studies using supported membranes

13:30-14:30 **Lunch & posters view. Foyer Elbkuppel**

14:30-16:00 **Poster flash talks and poster viewing. Foyer Elbkuppel**

Poster presenter give a talk with 1 slide to attract the audience to come to the poster and learn more about their research

16:00-17:00 **Second Plenary Talk. Elbkuppel**

Chair: Frederic Meunier
Jacqueline Burre (Weill Cornell Medicine, New York NY, USA)
Disease-causing mutations in Munc18-1/STXBP1 impair neurotransmitter release at multiple levels

17:00-17:30 **Afternoon coffee break and poster view. Foyer Elbkuppel**

17:30-19:30 **Evening session: Pathologies of the secretory machine. Elbkuppel**

Chairs: Sushil K. Mahata and N.N.

17:30 Manu Sharma (Weill Cornell Medicine, New York NY, USA)
Lysosomal exocytosis releases pathogenic α-synuclein species from neurons

18:00 Geert van den Bogaart (University of Groningen, Groningen, The Netherlands)
Regulation of syntaxin-5 function by alternate sites of translation initiation

18:30 Uri Ashery (Tel Aviv University, Tel Aviv, Israel)
Novel platform for detection of alpha-synuclein aggregation from skin biopsy using super-resolution microscopy

19:00 Sushil K. Mahata (University of California San Diego, USA)
Chromogranin A regulation of age-associated development of hypertension, diabetes, and dementia

19:30 **Free time, dinner & discussions at leisure**

Saturday 9th ISCCB-21

09:15-11:30 **Excursion: Harbor cruise including historic warehouse city**

Location: Landungsbrücken Pier 10 (very close to the conference hotel)

The world's largest warehouse complex is a UNESCO World Heritage Site and a must-see on every trip to Hamburg. But this cruise will show you much more of Hamburg's harbor and its ships and vessels. We will see part of the historic warehouse city, the newly built Hafencity, channels and locks, the Elbphilharmonie, Container Terminals, Docks, Ship repair facilities and of, course, views of our conference hotel from the water.

12:30 **ISCCB office opens. Foyer Elbkuppel**

13:00-14:00 **Lunch and posters view. Foyer Elbkuppel**

14:00-15:00 **Third Plenary Talk. Elbkuppel**

Chair: Josh Zimmerberg

14:00-15:00 Gary Whittaker (Cornell University, Ithaca NY, USA)
Activation of membrane fusion by the coronavirus spike protein

15:00-16:20 **Afternoon session: Viral fusion mechanisms. Elbkuppel**

Chair: Lukas Tamm

15:00-15:30 Josh Zimmerberg (National Institutes of Health, Bethesda MD, USA)
Role of cholesterol in the tug of war between viral fusion proteins and cellular restriction factors in virus entry hydration, promoting poration.

15:30-16:00 Lukas Tamm (University of Virginia, Charlottesville, VA, USA)
Role of cholesterol in the tug of war between viral fusion proteins and cellular restriction factors in virus entry

16:00-16:20 Borut Furlani (University of Ljubljana, Slovenia)
Monitoring SARS-CoV-2 infection in human astrocytes

16:20-17:30 **Wine & cheese club: Fusion from SNAREs to viruses**

Discussion Time with snacks and drinks

17:30-19:30 **Evening Session: Chromogranin A and B: Maintenance of homeostasis and impacts on diseases**

Chairs: Sushil K. Mahata and Carmine Rocca

17:30-18:00 Sushil K. Mahata (University of California San Diego, USA) *Catestatin and its mimetics as potential therapies for hypertension, diabetes, and gut motility disorders*

18:00-18:30 Julia von Blume (Yale University, USA).
Liquid-liquid phase separation facilitates the biogenesis of secretory storage granules

18:30-19:00 Carmine Rocca (University of Calabria, Italy)
Novel insight on Chromogranin A cardiac mechanism of action: the role of Neuropilin-1

19:00-19:30 Sahar El Aidy (University of Groningen, Netherlands)
This presentation is supported by the Biochemical Society
Catestatin plays crucial roles in regulating the abundance of gut bacterial taxa with selective antimicrobial resistance

19:30 **Free time, discussions & dinner at leisure**

Sunday 10th ISCCB-21

05:00-8:30 Visit the nearby Fischmarkt (fish market) (~10 min walk).

This is a fun experience with plenty of opportunities for breakfast and to buy some souvenirs. Note the time: Fischmarkt is open Only on Sundays (5:00 AM to 9:30 AM).

08:30 **ISCCB office opens. Foyer Elbkuppel**

08:30 **Coffee, Tea and Snacks, Foyer Elbkuppel**

9:00-11:00 **Early morning session: Lipids in the life cycle of secretory granules**

Chair: Nicolas Vitale

9:00-9:30 Emeline Tanguy (Université de Strasbourg, Strasbourg, France)
The multiple function of different phosphatidic acid species along the different steps of neuroendocrine secretion.

9:30-10:00 Yongsoo Park (Hamad Bin Khalifa University, Doha, Qatar)
Requirement of cholesterol for calcium-dependent vesicle fusion by stabilizing synaptotagmin-1-induced membrane bending

10:00-10:30 Luis Gutierrez (Universidad Miguel Hernández Alicante, Spain)
The sphingosine analogue FTY-720 influences membrane fusion and cell survival in chromaffin cells

10:30-11:00 Isaac Akefe (University of Queensland, Brisbane, Australia)
Greasing the wheels of memory with myristic fatty acids

11:00 **Coffee Break & Posters view**

11:30-13:00 **Late morning sessions (2 parallel)**

Ellipse **From chromaffin granules towards clinical applications**	**Elbkuppel** **Vesicle priming and recycling**
Chair: Ricardo Borges	Chair: Liz Seward
11:30 Ricardo Borges (University of La Laguna, Tenerife, Spain) *A secretory vesicle failure in Parkinson's disease occurs in human platelets*	11:30 Jakob Sørensen (Univ. of Copenhagen, Denmark) *ubMunc13-2, synaptotagmin-7 and phorbolesters/ diacylglycerol: a triad for dense-core vesicle priming.*
12:00 Antonio G. García (Universidad Autónoma de Madrid, Spain) *Altered exocytosis and mitochondrial function in chromaffin cells from the SOD1^{G93A} mouse model of amyotrophic lateral sclerosis in chromaffin cells*	12:00 Liangwei Gong (Univ. of Illinois, Chicago IL, USA) *Roles of sphingosine kinase 1 in synaptic vesicle recycling*
12:30 Andy Ewing (Gothenburg Univ., Gothenburg, Sweden) *Repetitive Stimulations, a new paradigm for understanding the role of partial exocytosis in plasticity*	12:30 Matthijs Verhage (CNCR Amsterdam, The Netherlands) *Organizing release sites for secretory vesicle exocytosis in chromaffin cells and neurons*

13:00 **Lunch & Posters view. Foyer Elbkuppel**

14:00-14:50 **Fourth Plenary Talk. Elbkuppel**
Chair:
Ling-Gang Wu (National Institutes of Health, Bethesda MD, USA)
Visualizing membrane dynamics of exo- and endocytosis

15:00-17:00 **Afternoon Sessions (2 Parallel Sessions)**

Elbkuppel **Exocytosis beyond chromaffin cells**	**Ellipse** **Ion Channels, Transporters, and Receptors**
Chair: Robert Zorec & Vladimir Parpura 15:00 Vladimir Parpura (University of Alabama at Birmingham, Birmingham AL, USA) *Vesicle docking: Mechanical interactions between SNARE proteins revealed by atomic force spectroscopy* 15:30 Manfred Frick (University of Ulm, Germany) *Exocytotic release of surfactant from the alveolar type II lung cells in health and disease* 16:00 Jernej Jorgačevski (University of Ljubljana, Slovenia) *Fusion pore stabilization by cholesterol in astrocytes and pituitary cells; relevance for lysosomal storage disease* 16:30 Robert Zorec (University of Ljubljana, Slovenia) *Heterologous lysosomal fusion and the cell-based immunotherapy of prostate cancer*	Chair: Emilio Carbone and Constanza Maldifassi 15:00 Martin Heine (Johannes Gutenberg Univ. Mainz, Germany) *Dynamic organization of voltage gated calcium channels in the presynaptic membrane* 15:30 Emilio Carbone (University of Torino, Italy) *Chromaffin cell firing viewed through MEAs: evidence for two distinct firing modes and well-resolved action potential-driven Na^+, K^+ and Ca^{2+} currents at the cell-microelectrode contact regio*n 16:00 Petronel Tuluc (University of Innsbruck, Austria) *Loss of α2δ-1 calcium channel subunit function increases excitability and promotes burst firing in mouse chromaffin cells* 16:30 Raul Guzman (Forschungszentrum Jülich, Jülich, Germany) *CLC anion/proton exchangers regulate secretory vesicle filling and granule exocytosis*

17:00-19:30 **Posters view with wine & beer. Foyer Elbkuppel**

17:00-18:00 **IAB Biennial Meeting**

18:00-19:00 **Special Workshop: Advanced Research Tools. Elbkuppel**
Chair:
Gert Rapp (Rapp OptoElectronic, Wedel, Germany)
High-performance photomanipulation and illumination systems, microscope modification, multi-photon-, spinning disk- and deep-UV microscopy and accessories.
Martin Oberhofer (HEKA Elektronik, Reutlingen, Germany)
Tools for Patch Clamp, Imaging and Capacitance Measurements
Steven Bump (Visitron Systems GmbH, Puchheim, Germany)
The VisiTIRF-ORBITAL Ring-TIRF technology

19:30 **Free time, discussions & dinner at leisure**

Monday 11th ISCCB-21

08:00 **ISCCB office opens. Foyer Elbkuppel**

08:30 **Coffee, Tea and Snacks**

09:00-11:05 **Early Career Researchers - *Daniel T. O'Connor, MD Memorial Session.* Elbkuppel**

Chairs: Nitish Mahapatra and Nicolas Vitale

9:00-9:25 Pradeep Kumar Singh (Rockefeller University, New York, USA)
Vascular contribution in Alzheimer's disease associated blood brain barrier damage, neuroinflammation and cognitive decline

9:25-9:50 Vikas Arige (University of Rochester, New York, USA)
Functional Determination of Calcium Binding Sites Required for the Activation of Inositol 1,4,5-trisphosphate receptors

9:50-10:15 Wei Ying (University of California San Diego, USA)
A CRIg+ macrophage-dependent defense against microbial extracellular vesicle-induced adrenomedullary dysfunctions

10:15-10:40 María Constanza Maldifassi (University of Bern, Switzerland)
Crosstalk between Pannexin1, P2X7 and α7 nicotinic acetylcholine receptors potentiate exocytosis

10:40-11:05 Carmine Rocca (University of Calabria, Italy)
A new predictive sensor for the doxorubicin-dependent cardiotoxicity: the paradigm of the cardioprotector chromogranin-A

11:05 **Coffee Break & Posters view. Foyer Elbkuppel**

11:30-13:00 **Morning sessions (2 parallel)**

Elbkuppel **Localization of the fusion machinery**	**Ellipse** **Microfabricated devices for studies of neurotransmission**
Chair: Nikhil Gandasi 11:30 Ute Becherer (Saarland University in Homburg, Germany) *CAPS1 and CAPS2 localization and function at dorsal ganglion root synapses* 12:00 Adekunle T Bademosi (QBI, University of Queensland, Brisbane QLD, Australia) *Super-resolving the molecular machinery underpinning fusion pore formation* 12:30 Wolf Almers (Oregon Health Sci. Univ., Portland OR, USA) *Syntaxin clusters and granules at the plasma membrane of cultured endocrine cells*	Chair: Valentina Carabelli 11:30 Manfred Lindau (University of Miami FL, USA) *A surprise from spatiotemporal analysis of simultaneous TIRF and electrochemical imaging of exocytic events in chromaffin cells* 12:00 Brian Kim (University of Central Florida, Orlando FL, USA) *1024-ch Amperometric Microelectrode Array for Accelerated Quantal Analysis from Human Neuroblastoma and PC-12 Cells* 12:30 Valentina Carabelli (University of Torino, Italy) *Micro-graphite patterned diamond sensors to monitor midbrain neurons activity and its alteration by a-synuclein*

13:00-14:00 Lunch & posters view

14:00-15:30 Afternoon sessions (2 parallel)

Elbkuppel **Kinetic aspects of transmitter release**	**Ellipse** **Chromogranin A and its peptides: Risk factors for cardiometabolic and gut disease**
Chair: Ira Milosevic and Meyer Jackson 14:00 Grant Kusick (Johns Hopkins Univ., Baltimore MD, USA) *Synaptotagmin 7 docks synaptic vesicles for Doc2α-triggered asynchronous neurotransmitter release* 14:30 Meyer Jackson (Univ. of Wisconsin, Madison WI, USA) *Rigid and flexible fusion pores of exocytosis* 15:00 Ira Milosevic (Univ. of Oxford, Oxford, UK) *Regulation of timing and efficacy of neurotransmitter release by the core complex associated protein complexin*	Chair: Elke Muntjewerff and Nitish Mahapatra 14:00 Nitish R. Mahapatra (Indian Institute of Technology Madras, India) *Functional human variants of catestatin and pancreastatin peptides enhance cardiometabolic disease risk* 14:30 Elke Muntjewerff (Uppsala University, Sweden) *The role of chromogranin A and catestatin in homeostasis and disease* 15:00 Ahmed Shaaban (University of Copenhagen, Denmark) *Dissecting the functional properties of serotonin release from "the other" chromaffin cells in the gut*

15:30 Coffee Break

16:00 Late afternoon Session: Super-resolution imaging - vesicles and the exocytotic machinery, Elbkuppel

Chairs : Ute Becherer and Uri Ashery

16:00-16:30 Fred Meunier (QBI, University of Queensland, Brisbane QLD, Australia)
Nano-biomolecular condensates control the clustering of synaptic vesicles

16:30-17:00 Justin Taraska (National Institutes of Health, Bethesda MD, USA)
Imaging the nanoscale structures of exocytosis and endocytosis with light and electron microscopy

17:00-17:30 Ali Shaib (University of Göttingen Medical Center, Göttingen, Germany)
Optimized Nanoscale Expansion Microscopy

17:30-18:00 Markus Sauer (Julius-Maximilians-Universität Würzburg, Germany)
Molecular resolution fluorescence imaging

18:00 Presentations of Awards Ceremony
Chairs: Uri Ashery and Liz Seward

18:30 Summing up & introducing the 22nd ISCCB

19:30 Walk to Station Landungsbrücken and take U3 to Rathaus (3 Stops)

20:00 Banquet (Restaurant "Parlament" in the Rathaus (City Hall)

https://www.parlament-hamburg.de/

With typical music by the Shanty Choir Fleetenkieker

Poster Presentations Friday-Monday

Rohith K. Nellikka, **Bhavya R. Bhaskar**, Kinjal Sanghrajka, Swapnali S. Patil, and Debasis Das
(Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai 400005, India)
α-Synuclein kinetically regulates the nascent fusion pore dynamics

Santiago Echeverry, Per-Eric Lund, Jan Saras, Johan Dunevall, and Sebastian Barg.
(Uppsala University, Department of Medical Cell Biology (MCB). Uppsala, Sweden)
Munc13 regulates exocytosis by two distinct Ca^{2+}-dependent mechanism.

Liangwen Liu, Muhmmad Omar-Hmeadi, Irina Česnokova, and Sebastian Barg
(Medical Cell Biology, Uppsala University, Sweden)
Munc18 isoforms differentially support docking and priming of insulin granules

Julijan Vršnik[1], Mićo Božić[1], Matjaž Stenovec[1,2], Robert Zorec[1,2]
([1]Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1000, Ljubljana, Slovenia; [2]Celica Biomedical, Tehnološki park 24, 1000, Ljubljana, Slovenia)
Noradrenergic suppression of IFNg-induced lysosomal deposition of MHC class II molecules at the surface of rat astrocytes

Tamara Theiner, Noelia Jacobo-Piqueras, Nadine J. Ortner, Stefanie M. Geisler, Petronel Tuluc
(Department of Pharmacology and Toxicology, Institute of Pharmacy, University of Innsbruck, Austria)
$Ca_V1.3$ L-type Ca^{2+} channel modulates pancreatic β-cell electrical activity and survival.

Noelia Jacobo-Piqueras, Tamara Theiner, Stefanie M. Geisler, Petronel Tuluc
(Department of Pharmacology and Toxicology, Institute of Pharmacy, University of Innsbruck, Austria)
Molecular mechanisms responsible for the sexual dimorphism in pancreatic β-cell insulin release

Kinjal Sanghrajka, Swapnali Patil, Debasis Das
(Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai 400005, India)
Investigating the Role of Synaptobrevin2 dimers in functional SNARE complex assembly

Stefanie M. Geisler[1], Tamara Theiner[1], Noelia Jacobo-Piqueras[1], Marta Campiglio[2], Petronel Tuluc[1]
([1]Centre for Molecular Biosciences, Department of Pharmacology and Toxicology, University of Innsbruck, 6020 Innsbruck, Austria; [2]Institute of Physiology, Medical University Innsbruck, Innsbruck 6020, Austria.)
Genetic deletion of Stac2 adaptor protein alters electrical activity of mouse chromaffin cells

Noemí Socas, Judith Estévez-Herrera, Natalia Domínguez, Ricardo Borges, José David Machado.
(*Pharmacology Unit, Medical School. Universidad de La Laguna. Tenerife. Spain.*)
Newly synthesized chromaffin secretory vesicles are preferentially released.

Robin Pritchard[1], Iman Aolymat[1], Jeff Barclay[1], Elizabeth Seward[2]
([1] Department of Molecular Physiology and Cell Signalling, University of Liverpool, UK; [2] School of Biosciences, University of Sheffield, UK)
Analysis of a HSPB1 mutation in Charcot-Marie-Tooth disease using patch-clamp electrophysiology of bovine chromaffin cells

Neha Sinha[1], Anuma Pallavi[2] and **Nikhil R Gandasi[1,2,3]**
([1]Islet Research lab, Department of Molecular Reproduction, Development and Genetics (MRDG), Indian Institute of Sciences (IISc), Bengaluru-560012, India; [2] Unit of Metabolic Physiology, Shalgrenska Academy, Gothenburg University, Gothenburg, Sweden)
Glucose uptake in the endocrine pancreatic cells through glucose transporters

Ana De la Iglesia, Noemí Socas, José D. Machado, and Ricardo Borges
(Pharmacology Unit, Medical School. Universidad de La Laguna. Tenerife. Spain)
An integrated system for quantifying chromaffin cell secretion

Oscar J. Parada-Parra and Arturo Hernández-Cruz
(Cognitive Neuroscience Department, Institute of Cellular Physiology. Universidad Nacional Autónoma de México. Ciudad de México. Mexico)
Effect of reversible SERCA blockade on depolarization-induced CA exocytosis in chromaffin cells from normotensive Wystar Kyoto rats and Spontaneously Hypertensive Rats

Karolina Stepien, Junjie Xu, Xuewu Zhang, Xiaochen Bai, Josep Rizo
(University of Texas Southwestern Medical Center at Dallas TX, USA)
Neuronal SNARE assembly enlightened by cryo-EM structures of a synaptobrevin-Munc18-1-syntaxin-1 complex

J. Fernando Padín [(1)], Iago Méndez-López [(2)], Antonio G. García [(2)]
([1]Department of Medical Sciences (Pharmacology), School of Medicine, UCLM, Ciudad Real, Spain (jf.padin@gmail.com), [2]Instituto Teófilo Hernando and Department of Pharmacology and Therapeutics, School of Medicine, UAM, Madrid, Spain)
Impairment of cellular maturation dynamics in early stages of ALS lead to alterations of exocytosis in the SOD1^{G93A} mouse

Federica Castellani[1,2,*], María Laura Fernández[3,4], Marcelo Risk[5], P. Thomas Vernier[1]
([1]Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk, VA 23508, USA; [2]present address:Univ. Miami, Miller School of Medicine, Dept. Physiology & Biophysics, Miami FL, USA;
[3]CONICET- Universidad de Buenos Aires. Instituto de Física del Plasma (INFIP). Buenos Aires, Argentina; [4]Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales. Departamento de Física. Buenos Aires, Argentina; [5]Instituto de Medicina Traslacional e Ingenieria Biomedica (IMTIB) CONICET-Instituto Universitario del Hospital Italiano de Buenos Aires, Argentina); * Present address: Univ. Miami Medical School, Miami FL, USA
Comparison of different force fields in MD simulations of ion-membrane interactions

Wonchul Shin[1*], **Chung Yu Chan[1*],** Lisi Wei[1], Lihao Ge[1], Weidong Zhao[1,2], Edaeni Hamid[1], Gianvito Arpino[1], Xiaoli Guo[1], Hsueh-Cheng Chiang[1,3], and Ling-Gang Wu[1] *Equal contribution
[1]National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA; [2]Current address: Department of Developmental Cell Biology, China Medical University, Shenyang, China; [3]Current address: Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan city, Taiwan;
Establishing a new fission model: Continuous dynamin constriction cuts through membrane neck

Chad P. Grabner and Tobias Moser
(Institute for Auditory Neuroscience, University Medical Center Göttingen, Germany; Auditory Neuroscience & Synaptic Nanophysiology Group, Max Planck Institute for Multidisciplinary Sciences, 37077 Göttingen, Germany
Collaborative Research Center 1286, University of Göttingen, Göttingen, Germany**;** Cluster of Excellence "Multiscale Bioimaging: from molecular machines to networks of excitable cells", University of Göttingen, Germany)
3D-MINFLUX nanoscopy and membrane capacitance measurements reveal how the mouse rod ribbon builds a large active zone

Vikas Arige*, Lara E. Terry*, Larry E. Wagner 2nd*, Mariah R. Baker[#], Guizhen Fan[#], Irina I. Serysheva[#], David I. Yule*
(*Department of Pharmacology and Physiology, University of Rochester, Rochester, NY, USA; [#]Department of Biochemistry and Molecular Biology, McGovern Medical School, The University of Texas Health Science Center, Houston, TX USA)
Functional Determination of Calcium Binding Sites Required for the Activation of Inositol 1,4,5-trisphosphate receptors

Andrea Marcantoni[1], Giuseppe Chiantia[2], Giulia Tomagra[1], Enis Hidisoglu[1], Valentina Carabelli[1], **Emilio Carbone**[1]
([1]Department of Drug Science, N.I.S. Centre, University of Torino, Italy, [2]Department of Neuroscience, University of Torino, Italy)
MEA recordings of spontaneously firing rat chromaffin cells uncover new high-frequency long-lasting bursts and well-resolved AP-driven Na^+, K^+ and Ca^{2+} currents sustaining the firing

Shailendra Singh Rathore[1], **Manfred Lindau[1,2]**
[1]School of Applied & Engineering Physics, Cornell University, Ithaca NY, USA; [2]Department of Physiology &Biophysics, University of Miami, Miller School of Medicine, Miami FL, USA
Spatiotemporal analysis of simultaneous TIRF and electrochemical imaging of exocytic events in chromaffin cells reveals "apparent" foot signals

A day to experience some of Hamburg's Architectural and Cultural Highlights

No Fee: Walk under the Elbe River through the historic Old Elbe Tunnel to the other side and enjoy the view of our conference location, the harbor landing piers and the entire City of Hamburg.
https://www.hamburg-travel.com/see-explore/maritime-hamburg/old-elbe-tunnel/

No Fee: Walk through the nearby newly developed Hafencity and take a tour of the Elbphilharmonie
https://www.elbphilharmonie.de/en/visit

No Fee: There are several ferry boats from Landungsbrücken, which are part of the local public transport. Take your 9 € ticket and enjoy the ride.

No Fee but ticket booking required:
A panoramic view of the port and the city from a height of 37 meters above ground level from the Elbphilharmonie Plaza Viewing Platform
Concert Hall Tours in English are offered for 20.00 €

No Fee: Check out the traditional ship harbor in the Hafen City https://www.hamburg-travel.com/see-explore/maritime-hamburg/traditional-ship-harbour-in-the-sandtorhafen/

or the international maritime museum https://www.hamburg-travel.com/see-explore/culture-music/museums-galleries/international-maritime-museum/

No Fee: Take a trip to Schulau (Schulauer Fährhaus) down the Elbe River where all arriving and departing ships are greeted with their National Anthem. There is currently no boat line going to this point but with your 9 € ticket you can take S1 from Landungsbrücken to Wedel, then bus 189 direction "Blankenese" after 4 min exit at "Wedel Elbstrasse (Willkomm Höft)" It is 5-8 min walk from here.
https://www.schulauer-faehrhaus.de/
https://en.wikipedia.org/wiki/Willkomm-Höft

Admission Fee required: Visit the largest model railway of the world
https://www.miniatur-wunderland.com/

Buy a theater ticket to watch a Musical in a nearby Theater:
At the following web site you can book in English

https://www.hamburg-travel.com/booking/tickets/musicals/

The Beatles Musical "All you need is love" will be shown in the St. Pauli Theater.
https://www.st-pauli-theater.de/programm/all-you-need-is-love-2/

The Tina Turner Musical at Operettenhaus
https://www.stage-entertainment.de/musicals-shows/tina-hamburg

The Lion King (König der Löwen) across the Elbe river (special Ferry included)
https://www.stage-entertainment.de/musicals-shows/disneys-der-koenig-der-loewen-hamburg

ISCCB-21 List of Participants

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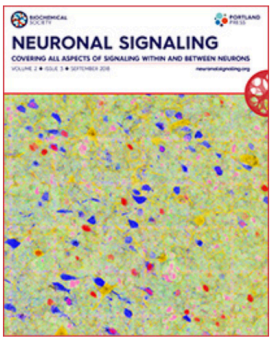

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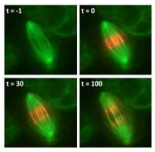

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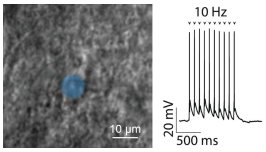

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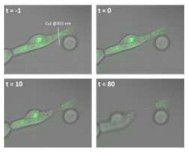

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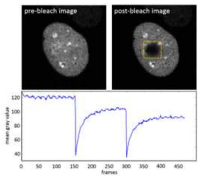

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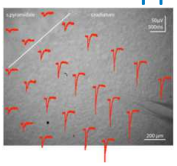
Neuronal mapping



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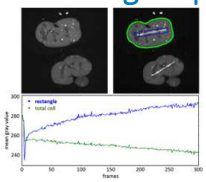

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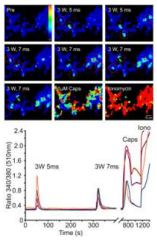
Heat shock studies



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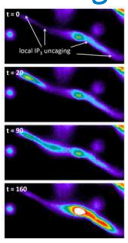

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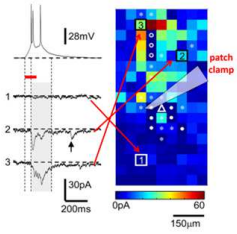
Glu uncaging



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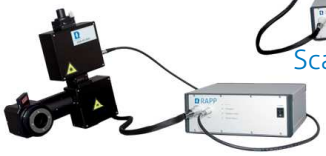

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ISCCB-21

Abstracts

Intermediate steps in SNARE-mediated membrane fusion

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It is generally accepted that SNARE proteins mediate membrane fusion by directed assembly of complementary SNARE motifs between the membranes destined to fuse (SNARE zippering). While SNARE-zippering overcomes the repulsive forces between the membranes, it is not known in which way SNAREs are involved in the subsequent transition states and how they affect the energy barriers along the reaction coordinate of fusion. For instance, in neurons synaptic vesicles are maintained in a docked and primed state before exocytosis, requiring only calcium activation of synaptotagmin to elicit fusion, but the state of SNARE assembly and the precise role of the accessory proteins is debated. In the lecture I will discuss possible scenarios for the structure and stabilization of the docked and primed state and the subsequent transition towards non-bilayer intermediates along the fusion pathway.

Syntaxin-1A modulates vesicle fusion in mammalian neurons via juxtamembrane domain dependent palmitoylation of its transmembrane domain

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SNAREs are undoubtedly one of the core elements of synaptic transmission. On the contrary to the well characterized function of their SNARE domains bringing the plasma and vesicular membranes together, the level of contribution of their juxtamembrane domain (JMD) and the transmembrane domain (TMD) to the vesicle fusion is still under debate. To elucidate this issue, we analyzed three groups of STX1A mutations: 1) elongation of STX1A's JMD by three amino acid insertions in the junction of SNARE-JMD or JMD-TMD; 2) charge reversal mutations in STX1A's JMD; and 3) palmitoylation deficiency mutations in STX1A's TMD. We found that both JMD elongations and charge reversal mutations have position dependent differential effects on Ca^{2+}-evoked and spontaneous neurotransmitter release in cultured murine hippocampal neurons. Importantly, we show that STX1A's JMD regulates the palmitoylation of STX1A's TMD and loss of STX1A palmitoylation either through charge reversal mutation K260E or by loss of TMD cysteines particularly inhibits spontaneous vesicle fusion. Interestingly, the retinal ribbon specific STX3B has a glutamate in the position corresponding to the K260E mutation in STX1A and mutating it with E259K acts as a molecular on-switch. Furthermore, palmitoylation of post-synaptic STX3A can be induced by the exchange of its JMD with STX1A's JMD together with the incorporation of two cysteines into its TMD. Forced palmitoylation of STX3A dramatically enhances spontaneous vesicle fusion suggesting that STX1A regulates spontaneous release through two distinct mechanisms: one through the C-terminal half of its SNARE domain and the other through the palmitoylation of its TMD.

Molecular mechanisms underlying the exquisite regulation of neurotransmitter release

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The release of neurotransmitters by Ca^{2+}-evoked synaptic vesicle exocytosis is tightly regulated by a sophisticated protein machinery. Extensive research has led to a model whereby formation of SNARE complexes between the SNARE motifs of syntaxin-1, SNAP-25 and synaptobrevin is crucial for exocytosis. These complexes are disassembled by NSF and SNAPs, and are assembled in an NSF-SNAP-resistant manner by Munc18-1 and Munc13-1, enabling regulation of release in a variety of presynaptic plasticity processes that depend on Munc13-1 and associated proteins. Upon assembly, synaptotagmin-1 and complexin-1 bind to the SNARE complex, forming a primed state that is ready to induce fast membrane fusion upon Ca^{2+} binding to synaptotagmin-1. However, crucial questions remain about the mechanism of SNARE complex assembly and about the nature of the primed state. Building on previous work suggesting that Munc18-1 binds to syntaxin-1 folded into a self-inhibited closed conformation and later binds also to synaptobrevin, forming a template to assemble the SNARE complex, we have elucidated two cryo-electron microscopy structures of Munc18-1 bound to the SNARE complex. The structures reveal how the syntaxin-1 N-terminal region plays a key role in initiating SNARE complex assembly. Using all-atom molecular dynamics simulations, we find that the SNAREs alone have a tendency to induce formation of extended membrane-membrane contact interfaces that fuse slowly. Our data also suggest that synaptotagmin-1 and complexin-1 hinder formation of these interfaces and, together with the SNARE complex, form a spring-loaded macromolecular assembly that hinders premature fusion but is ready for fast fusion upon Ca^{2+} binding to synaptotagmin-1.

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Dynamic Priming in both Chromaffin cells and Synaptic Terminals

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When studying secretion from both chromaffin cells and Calyx of Held nerve terminals with similar techniques a puzzle emerged initially. In both preparations cells were stimulated strongly by either voltage-clamp depolarization or else flash-photolysis of caged-Ca. Release was measured on the single-cell level by either membrane capacitance or postsynaptic current. In both systems rapid bursts of release could be observed consisting of fast and slow components. The puzzle was that in chromaffin cells the amplitudes of such responses to pool-depleting stimuli very much depended on the level of cytoplasmic Ca^{++}-concentration ($[Ca^{++}]$) before the stimulus, while at synapses responses seemed to be pretty robust with respect to such manipulations. Nevertheless, many similarities regarding the involvement of secretory proteins in the control of exocytosis were noted, when comparing both preparations (Rettig & Neher, 2002, Science **298:**781-785; Walter et al., 2011, Trends Neurosci **34**:487-497; Neher, 2018, Eur J Physiol **470**:7-11). However, "Dynamic Priming", i.e. the dependence of the "Readily Releasable Pool" (RRP) of secretory vesicles on levels of second messengers, such as $[Ca^{++}]$, and on the repertoire of secretary proteins (Tawfik et al, 2021, **eLife** 10:e64527), seemed to be a specialty of catecholamine release.

More recently it turned out that this is not at all the case. Rather, it seems that much of so-called "synaptic plasticity" may be the consequence of such regulating processes, which happen at rest. A key finding was that the Calyx of Held is quite special in its robustness against changes in resting $[Ca^{++}]$. At other glutamatergic synapses it could be shown that under standard conditions sites of neurotransmitter release are only partially occupied by docked vesicles and that such occupancy can be changed by manipulating $[Ca^{++}]$ (Malagon et al, 2020, eLife **9**:e52137). Also, it turned out that a large stable RRP requires the presence of specific isoforms of synaptic proteins (He et al, 2017, Nat Commun 8:15915). Furthermore, flash-and-freeze electron microscopy showed that synaptic vesicles can be observed in two states of docking at the plasma membrane – loose and tight – and that neuronal activity transiently increases the number of tightly docked synaptic vesicles (Kusick et al. Nat Neurosci **23**:1329-1338).

Consequences of these findings for synaptic plasticity in terms of a two-state priming model were discussed by Neher & Brose (2018), Neuron **100**, 1283-1291. A quantitative analysis of synaptic responses in terms of such a model was able to explain many aspects of glutamate release. So, in the end, work on catecholamine secretion may tell us not only about neurotransmitter release *per se*, but also about synaptic plasticity.

Molecular mechanisms of calcium triggered exocytosis

Axel Brunger

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The central nervous system relies on electrical signals traveling along neurons at high speeds. Signals are also transmitted between two neurons, or from a neuron to a muscle fiber through synaptic junctions. Synaptic transmission relies on the release of neurotransmitter molecules into the synaptic cleft. This release in turn depends on a process called membrane fusion to ensure that the neurotransmitter molecules that are contained in synaptic vesicles are released into the synaptic cleft as quickly as possible. Membrane fusion is an important process in many areas of biology, including intracellular transport and hormone release, but it occurs much faster (< 1 millisecond) for synaptic vesicle fusion than for these other processes. Moreover, it is precisely calcium regulated. Moreover, similar proteins are involved in other calcium triggered fusion processes, such as mucin secretion. Recent studies of the molecular mechanisms of calcium triggered exocytosis will be presented.

Energetics and kinetics of chaperoned SNARE assembly revealed by optical tweezers

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Fusion of transmitter-containing vesicles with plasma membranes at the synaptic and neuromuscular junctions mediate neurotransmission and muscle contractions, respectively, thereby underlying all thoughts and actions. The fusion process is driven by the coupled folding and assembly of *three* synaptic SNARE proteins - syntaxin-1 and SNAP-25 on the target plasma membrane (t-SNAREs) and VAMP2 on the vesicular membrane (v-SNARE). Their assembly is chaperoned by Munc18-1 and many other proteins to achieve the speed and accuracy required for fusion. However, the physiological pathway of SNARE assembly remains unclear. Here, we report our recent progress in understanding SNARE assembly and its associated intermediates, energetics, and kinetics mainly derived from *single*-molecule manipulation approaches. Assembly of the three SNAREs begins with the formation of a t-SNARE binary complex, on which VAMP2 folds in a zipper-like fashion. The t- and v-SNAREs zipper stepwise via multiple partially zippered states, drawing the two membranes to fuse via distinct stages. Recent structural and single-molecule studies, however, suggest an alternative pathway: syntaxin-1 and VAMP2 first bind on the surface of Munc18-1 to form a template complex, with which SNAP-25 associates to conclude SNARE assembly. We characterize the structure and stability of the template complex and its role in Munc18-1-chaperoned SNARE assembly and membrane fusion. Together, these results demonstrate that stepwise SNARE assembly drive stagewise membrane fusion.

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A molecular mechanism for Ca^{2+} triggered vesicle fusion during regulated exocytosis

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It has long been known that synatpotagmin-1 (Syt1) is the sensor that triggers fast, evoked release of neurotransmitter by the fusion of synaptic vesicles to the presynaptic membrane in response to calcium. It is also well established that the SNAREs syntaxin-1a, SNAP-25, and synaptobrevin-2/VAMP-2 form the core of the membrane fusion machinery that drives calcium-triggered neuronal exocytosis. We recently proposed a mechanism where the lipid bilayer is intimately involved in coupling calcium sensing to fusion. Using TIRF- (total internal reflection fluorescence) and sd-FLIC (site-directed fluorescence interference contrast) microscopy, we demonstrate that fusion of dense core vesicles purified from PC12 cells and insulin granules purified from INS1 cells with supported membranes containing syntaxin-1a and SNAP-25 correlates with the conformation (tilt angle) of the nascent SNARE complex on the target membrane. As the tilt angle increases, force is exerted on the SNARE transmembrane domains to drive the merger of the two bilayers as the trans-SNARE complex completes folding into the cis-SNARE complex. The SNARE conformation is modulated by the order of the lipid bilayer, and the order of the bilayer is changed by Ca^{2+} dependent binding of the two C2 domains of Syt1, linking SNARE conformation to C2 domain bilayer interactions. The strong dependency of Ca^{2+} dependent vesicle fusion efficiency on membrane order is further confirmed in live INS1 cells where the plasma membrane is enriched with lipids of defined acyl-chain saturation.
In addition to fusion efficiencies and fusion kinetics, TIRF data from single vesicle fusion events contains information about how the fluorescent content is released. Of particular interest is how fast the content is released. We present data that show how interactions between PIP2 and conserved arginine residues of Syt1's C2B domains regulate SNARE mediated fusion pore expansion.

Disease-causing mutations in Munc18-1 / STXBP1 impair neurotransmitter release at multiple levels

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Munc18-1 facilitates neurotransmitter release at the presynaptic terminal via its interactions with neuronal SNARE proteins, Doc2A/B, rab3, and Mint1/2. Heterozygous de novo mutations in the neuronal protein Munc18-1/STXBP1 are linked to epilepsies, intellectual disability, movement disorders, and neurodegeneration. These devastating diseases have a poor prognosis and no known cure, due to lack of understanding of the underlying disease mechanism. To determine how mutations in Munc18-1 cause disease, we used C. elegans models, Munc18-1 knockout mouse neurons expressing wild-type or mutant Munc18-1, hemizygous Munc18-1 mice, as well as in vitro studies. We found that at least five disease-linked missense mutations of Munc18-1 result in destabilization and aggregation of the mutant protein. Aggregates of mutant Munc18-1 incorporate wild-type Munc18-1, depleting functional Munc18-1 levels beyond hemizygous levels. We furthermore found that missense mutations or reduced levels of Munc18-1 trigger instability and aggregation of syntaxin-1, Doc2A, and Doc2B. We propose a model where the impact of mutant Munc18-1 on its effectors may give an insight into how these mutations cause such varying symptoms and severity, and suggest that different cell types experience different impairments resulting from the specific expression patterns of Munc18-1 effectors within the brain.

Lysosomal Exocytosis Releases Pathogenic α-Synuclein Species from Neurons

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Considerable evidence supports the release of pathogenic aggregates of the neuronal protein α-Synuclein (αSyn) into the extracellular space. While this release is proposed to instigate the neuron-to-neuron transmission and spread of αSyn pathology in synucleinopathies including Parkinson's disease, the molecular-cellular mechanism(s) remain unclear. Here we show that pathogenic species of αSyn accumulate within neuronal lysosomes in mouse brains and primary neurons. We then find that neurons release these pathogenic αSyn species via SNARE-dependent lysosomal exocytosis; proposing a central mechanism for exocytosis of aggregated and degradation-resistant proteins from neurons.

Regulation of syntaxin-5 function by alternate sites of translation initiation.

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The SNARE protein syntaxin-5 is an essential SNARE for Golgi transport. In humans, the STX5 gene encodes two isoforms (Stx5 Short and Long) that result from alternative starting methionines at position 1 (Long) and 55 (Short). We identified a novel human disorder caused by a single nucleotide mutation in this gene, resulting in the loss of the short isoform. Patients carrying this mutation suffer severe pathologies, including abnormal N-glycosylation, skeletal disorders and very short survival (<3 months). Using a novel functional microscopy technique, we show with patient-derived fibroblasts (only long isoform) and a CRISPR-engineered cell model with the long isoform selectively removed (only short isoform) that Stx5 Short is the dominant SNARE for intra-Golgi transport. Thus, we provide the cellular mechanism of why the long isoform of syntaxin-5 is unable to rescue the deficiency of the short isoform in the patients. This is the first mutation in an alternative starting codon causing a human disease, which provides the first functional evidence that proteins are regulated at the level of translation.

Reference:
Linders, P.T.A., Gerretsen, E., Ashikov, A., Vals, M.A., de Boer, R., Revelo, N.H., Arts, R., Baerenfaenger, M., Zijlstra, F., Huijben, K., Raymond, K., Muru, K., Fjodorova, O., Pajusalu, S., Õunap, K., ter Beest, M., Lefeber, D., & van den Bogaart, G. (2021) Congenital disorder of glycosylation caused by starting site-specific variant in syntaxin-5. Nature Communications. 12: 6227

Detection of Alpha-Synuclein Aggregates in Skin Biopsies of Parkinson's Disease Patients Using Super Resolution Microscopy

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Alpha-synuclein (a-Syn) aggregates in the central nervous system (CNS) have been associated with the development of Parkinson's Disease (PD) and are currently its main pathological hallmark. A-Syn aggregation has been also identified in many peripheral tissues such as skin, suggesting a novel and accessible method for the detection of the disease.
The main goal of this research was to establish a-Syn in skin biopsies as a comparative biomarker for the detection of PD pathology by utilizing super-resolution microscopy (SRM). This unique method, and specifically the Direct Stochastic Optical Reconstruction Microscopy (*d*STORM) that we applied, provides the opportunity to investigate the composition of a-Syn aggregation, and perhaps might overcome the challenge to identify small (sub-diffraction) a-Syn aggregates already in early stages of their formation.
Here, using SRM, we imaged phosphorylated and total a-Syn in immunostained sweat glands of skin biopsies from volunteering PD patients and control subjects, which were recruited and clinically assessed at Tel-Aviv Sourasky Medical Center and Meir Hospital. We applied a series of cluster analysis algorithms (DBSCAN & HDBSCAN) on the SRM output to characterize the precise size, shape, density, and composition of a-Syn aggregates and their distribution within the sweat glands' innervation, in order to explore possible correlations between the structural classifications and presence of the disease. We will describe preliminary results and the characterization off novel a-Syn aggregation in skin biopsies. This novel method may establish sub-diffraction a-Syn aggregates as a new biomarker for PD and may be used to explore early changes in the prodromal stage of PD, by identifying individuals at risk years before PD onset.

Chromogranin A regulation of age-associated development of hypertension, diabetes, and dementia

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Background: Aging-related diseases and disorders are a public health, societal, and economic problem around the world. Adults aged 65 years and older are the fastest growing segment of the US population. Cognitive impairment is among the most feared aspects of growing old as it heralds dementia (loss of memory), illness, and death. Dementia is the final stage of cognitive deterioration and is present in 5% of the population over 65 years of age. Hypertension and diabetes are also highly prevalent in the aging population. Feeding triple transgenic Alzheimer's disease mice, a high fat diet for 9 months worsens memory deficits, which links metabolism to the loss of memory. Hypertension and diabetes are both associated with the onset of vascular dementia and Alzheimer's disease dementia. Increased Chromogranin A (CgA) expression is found in Aβ plaques and tau tangles, both of which are hallmarks of Alzheimer's disease, suggesting a role for CgA in these pathologies. Therefore, we have determined the status of hypertension, diabetes, and dementia in aging CgA knockout (CgA-KO) mice.

Results: Young CgA-KO mice displayed two opposite aging phenotypes: hypertension but heightened insulin sensitivity. In comparison, aging WT mice gradually lost glucose tolerance and insulin sensitivity. Moreover, while aging WT mice had increased inflammation with higher plasma TNF-α, IFN-γ, and CCL2 along with increased mitochondrial fission, these phenotypes were the opposite in aging CgA-KO mice. CgA-KO mice also showed increased expression of mitochondrial and nuclear-encoded complex I genes in the liver, implying better mitochondrial function than WT mice. Most intriguingly, the hypertension in CgA-KO mice was spontaneously reversed with aging and they lived at least 40 months compared to 28 months of longevity in WT mice. Moreover, aging WT mice show increased expression of anti-insulin CgA-derived peptide pancreastatin but decreased expression of pro-insulin CgA-derived peptide catestatin. Consistent with these findings, supplementation of aging CgA-KO mice with pancreastatin increased both blood glucose levels and blood pressure, implicating pancreastatin as the causative factor in the development of hyperglycemia and hypertension in aging mice. Furthermore, we found that ablation of the CgA gene in tauopathy PS19 mice ($CgA^{-/-}$/$PS19^{+/-}$) resulted in dramatic decrease in expression of phosphorylated Tau (Ser202 by using CP13 antibody; Ser202/Thr205 by using AT8 antibody; Ser396/Ser404 by using PHF1 antibody), which possibly explains the increased longevity in $CgA^{-/-}$/$PS19^{+/-}$ mice.

Conclusion: We conclude that age-associated development of hypertension and diabetes are caused by CgA and thereby CgA is an aging-inducing factor.

Activation of membrane fusion by the coronavirus spike protein

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Coronaviruses (CoVs) contain a class I fusion protein (the spike protein, or S) that has both common features and distinct properties when compared to the prototypical viral fusogen influenza HA. Both S and HA are activated by host-cell proteases, such as TMPRSS2, to expose their fusion peptide (FP). However, many coronaviruses are also primed by a second cleavage event, often via the proprotein convertase furin, which can act as a key regulator of viral transmission and pathogenesis. Coronaviruses also demonstrate much more versatility in the range of proteases that can mediate priming and activation, which results is a much broader infection profile in terms of virus entry pathway (endosomal vs. cell surface), and in cell- and tissue-tropism. While the HA fusion peptide is “external” - in that the cleavage event exposes a key N-terminal glycine (G) residue on the FP - the CoV FP is “internal”, and consists of two domains (FP1 and FP2/FPPR) with a critical LLF motif in FP1 and series of negatively-charged residues that create a binding site for Ca^{2+} ions. We present a comprehensive study of the FP from SARS-CoV-1, MERS-CoV and SARS-CoV-2 using a range of biophysical and cell biological techniques, and demonstrate the utilization of fusion activation for the development of broad-based therapeutics and vaccines for use in treating respiratory diseases.

Aggregation of the fusion peptide of influenza HA alters membrane structure and hydration to promote poration

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Enveloped viruses feature surface spike proteins that mediate membrane fusion, such as the hemagglutinin (HA) of influenza virus. Upon activation, a domain of HA extends towards the target cell and embeds at its N-terminal tip a 21 amino acid amphipathic alpha helix, the fusion peptide (FP) critical for fusion. Synthesized FP of influenza virus are membrane active, by themselves generating pores in giant unilamellar vesicles (GUV). This activity can potentially explain both influenza virus' hemolytic activity and the liposome poration we saw in cryo-electron tomography. Fluorescent labeled FP was equally active on GUV, with increased heterogeneity at the time of poration. Molecular dynamics (MD) simulations of asymmetric bilayers with different numbers of FP in one leaflet show substantial FP clustering. At the center of this FP condensate, a profound change in the membrane structure results in thinning, higher water permeability, and curvature. Ultimately, a hybrid bilayer domain can form with one lipid leaflet and one FP leaflet. Membrane elastic theory predicts a reduced barrier to water pore formation when even a dimer of FP thins the membrane as above, and the FP tilt to initiate the required leaflet bending.

Role of cholesterol in the tug of war between viral fusion proteins and cellular restriction factors of viral entry

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To enter cells and establish an infection, viruses fuse their lipid envelopes with plasma or endosomal membranes of host cells. The refolding of viral fusion proteins provides the energy to perform work on the membranes, connect them, and bend them into fusion pores. Cholesterol stiffens and thickens lipid bilayers and can induce liquid ordered/disordered phase separation in model and biological membranes, which in turn produces line tension at the phase boundaries. All of these factors alter the energy profile of viral and other membrane fusion reactions.

Using single particle fusion experiments, we previously showed that cholesterol shifts the proportion of full fusion over stalled hemifusion in favor full fusion in viral and SNARE-mediated fusion reactions, meaning that cholesterol facilitates completion of fusion. In Ebola virus fusion, cholesterol binds directly to the TM domain of the GP2 fusion protein, which also interacts directly with the fusion loop of GP2, as demonstrated by NMR and FRET experiments.

Fusion of HIV pseudoviruses is promoted by line tension at domain boundaries between ordered and disordered lipid phases in the cellular target membrane. Serinc5 is a host cell encoded integral membrane protein that incorporates into the viral envelope of HIV particles during virus budding in the absence of Nef, a viral protein that regulates HIV replication. Serinc5 and some of its homologues have been shown to restrict viral entry in a mechanism that is poorly understood. We show by a combination of single particle fusion assays and cryo electron tomography (cryo ET) with HIV receptor and coreceptor bearing cell membrasnes that Serinc5 restricts a very late stage of viral-cell membrane fusion, namely the final expansion of the fusion pore, thereby inhibiting capsid entry into the cell. When incorporated into the viral envelope, Serinc5 increases the membrane heterogeneity of the viral membrane as shown by cryo ET and order sensitive lipid dyes. Serinc5-induced increased membrane order and heterogeneity of the viral envelope reduces the probability of fusion pore expansion, but the high negative curvature-inducing lipid PE can counteract Serinc5's inhibitory function on fusion, indicating a delicate balance between fusion promoting and fusion restricting lipid factors that are strongly affected by the presence of the restriction factor Serinc5 in the viral membrane.

Monitoring SARS-CoV-2 infection in human astrocytes

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the ongoing global COVID-19 pandemic, exhibiting a number of neurological signs in patients. It is likely that SARS-CoV-2 infects neural cells directly, especially astrocytes, in which aerobic glycolysis is present, supporting virus replication. Here we studied whether SARS-CoV-2 infects human astrocytes, an abundant type of neuroglial cells in the human brain, and whether this can be halted by ketamine. Ketamine is an anesthetic, used to induce a loss of consciousness and relieve pain, including in COVID-19 patients in intensive care. Previously, we have demonstrated a novel effect of ketamine, which manifests as the stabilization of a narrow exocytotic fusion pore and an inhibitory effect on vesicle endocytosis. Ketamine may exert its effects on fusion pore activity directly, or indirectly, by modulating cholesterol domains in the plasmalemma of astrocytes. To enter cells, SARS-CoV-2 relies on its obligate receptor, angiotensin-converting enzyme 2 (ACE 2) and subsequent endocytosis. Therefore, we have addressed the question, whether ketamine treatment may serve as a potential ally to alleviate SARS-CoV-2 infection. To this end, human astrocytes, which were either treated with ketamine or not, were infected with two strains (B.1.1.1.7 and B.1.258.17) of SARS-CoV-2, respectively. By using confocal microscopy and structured illumination microscopy (SIM), we have assessed if ketamine affects the infection rate, by monitoring percentage of infected cells, as well as the expression and distribution of ACE-2 and two viral proteins (RNA-dependent RNA polymerase - RDRP and envelope protein - E) immune-cytochemically in SARS-CoV-2-infected astrocytes. While ketamine-treatment did not significantly affect the gradual decrease in the viability of SARS-CoV-2-infected astrocytes, the percentage of astrocytes, infected with B.1.258.17, was lower in ketamine-treated cells. Infection of astrocytes with either strain of SARS-CoV-2 upregulated the expression of ACE-2 and triggered its redistribution to the cell periphery, whereas ketamine was shown to partially attenuate this effect. Furthermore, the density of RDRP- and E-proteins was lower in ketamine-treated astrocytes, compared with the non-treated controls. In summary, our study on human astrocytes highlights the potential benefits of ketamine-treatment in COVID-19 hospitalized patients.

Catestatin and its mimetics as potential therapies for hypertension, diabetes, and gut motility disorders

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Background: Hypertension (high blood pressure) and diabetes (high blood glucose) are prevalent in aging populations. Nearly 70% of older adults have hypertension and diabetes represents the most common metabolic disease in older adults. In humans, dysmotility of the gut potentiates infections, causes nutrient malabsorption, and manifests with debilitating symptoms, such as gastroparesis, intestinal pseudo-obstruction, diarrhea, constipation, Hirschsprung's disease, and fecal incontinence. About 20% of older people experience chronic constipation. This results in failure to absorb nutrients and eliminate harmful metabolites, as well as increasing incidence of infection. These disorders profoundly affect health and quality of life. In the USA, gastrointestinal diseases cost $135.9 billion annually.
Gut motility is regulated by the coordinated activity of smooth muscle cells, pacemaker interstitial cells of Cajal, motor neurons in the enteric nervous system (aka the "brain of the gut"), and the parasympathetic and sympathetic nervous system. Acetylcholine, substance P, 5-hydroxytryptamine (5-HT) or serotonin (via 5-HT_4 receptor), and bacterial short-chain fatty acids (via 5-HT synthesis and release) are known to accelerate gut motility. On the other hand, gastroparesis, dopamine (via dopamine 2 receptor), vasoactive intestinal peptide, and nitric oxide delay gut motility.

The chromogranin A peptide catestatin (CST) acts as a short-term antagonist to the ACh receptor, inhibiting catecholamine secretion; and as a long-term agonist, stimulating ACh secretion and thereby could be expected to affect gut motility.

Results: We have recently shown that CST is produced in the enteroendocrine cells of the gut and regulates gut permeability, the gut innate immune system, and gut microbiome composition. We found delayed gastric emptying and gut motility in CST knockout (CST-KO) mice, which is coupled with: (i) enlarged stomach (gastroparesis), (ii) decreased butyrate, (ii) decreased 5-HT, (iii) increased norepinephrine, and (iv) increased dopamine content in the gut. Treatment of CST-KO mice with CST improved gastric emptying and gut motility. To further establish the therapeutic potential of CST, we have determined its pharmacokinetic properties after oral administration of ~17.19 nM CST: Bioavailability: 15 minutes; C_{max} (maximal plasma concentration): ~11.24 nM; T_{max} (time to reach maximal plasma concentration): 1 hour; T1/2: ~6.7 hours; plasma concentration after 24 hours of oral administration: ~4.36 nM as compared to ~0.6 nM after 24 hours of intraperitoneal administration; degradation of CST after 24 hours of oral administration: ~74.6%

Conclusions: We propose that CST will ameliorate hypertension and diabetes and improve gut dysmotility and might provide potential therapies for related diseases and disorders.

Liquid-liquid phase separation facilitates the biogenesis of secretory storage granules

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Abstract

Insulin is a key regulator of human metabolism, and its dysfunction leads to diseases such as type 2 diabetes. It remains unknown how proinsulin is targeted from the trans-Golgi network (TGN) to secretory storage granules as no cargo receptor has been identified. Chromogranin proteins (CGs) are central regulators of granule biogenesis, and it was proposed that their aggregation is critical for this process. However, the molecular mechanism by which these molecules facilitate sorting at the TGN is poorly understood. Here, we show that CGs undergo liquid–liquid phase separation (LLPS) at low pH independently of divalent cations, such as calcium. Liquid CG condensates, but not aggregates, recruit and sort proinsulin and other granule-destined cargo molecules towards secretory granules. Cargo selectivity is independent of sequence or structural elements but is based on the size and concentration of the client molecules at the TGN. Finally, electrostatic interactions and the N-terminal intrinsically disordered domain of chromogranin B facilitate LLPS and are critical for granule formation. We propose that phase-separated CGs act as a “cargo sponge” within the TGN lumen, gathering soluble client proteins into the condensate independently of specific sequence or structural elements, facilitating receptor-independent sorting. These findings challenge the canonical TGN sorting models and provide insights into granule biosynthesis in insulin-secreting β-cells.

Novel insight on Chromogranin A cardiac mechanism of action: the role of Neuropilin-1

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Chromogranin A (CgA), a 439-residue long protein, is a cardiovascular regulator generating various bioactive fragments. Under stressful/pathological conditions, CgA proteolytic cleavage produces the CgA1-373 proangiogenic fragment. Here, we investigated the possibility of human CgA1-373 to influence the mammalian cardiac performance focusing on the role of its C-terminal sequence.

On the *ex vivo* Langendorff rat heart model, CgA1-373 elicited direct dose-dependent negative inotropism and vasodilation, while CgA1-372, a fragment lacking the C-terminal R373 residue, was ineffective. Antibodies against the PGPQLR373 C-terminal sequence abrogated the CgA1-373 -dependent cardiac and coronary modulation, which were mediated by endothelium, neuropilin-1 (NRP1) receptor, Akt/NO/Erk1,2 pathways, NO production and S-nitrosylation. *In vitro* experiments on H9c2 cardiomyocytes indicated that CgA1-373 also induced eNOS activation directly by targeting NRP1, thus providing beneficial effects against isoproterenol-induced hypertrophy and brain natriuretic peptide release. Molecular docking and all-atom molecular dynamics simulations strongly supported the hypothesis that the C-terminal R373 residue of CgA1-373 directly interacts with NRP1. Preliminary data also indicate that CgA1-373, but not CgA1-372, exerted cardioprotection in a rat model of doxorubicin cardiotoxicity. On the other hand, a pilot study on breast cancer patients shows a negative correlation between troponin T and CgA1-372/373 plasma levels before or after the 2nd cycle of an anticancer regimen including anthracyclines.

The results suggest that CgA1-373 may be considered as a new cardioregulatory hormone and that the removal of R373 represents a critical switch for turning "off" its cardioregulatory activity. NRP1 has emerged as a crucial actor in CgA1-373 patho-physiological cardiac mechanism of action.

Catestatin selects for colonization of antimicrobial-resistant gut bacterial communities

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The gut microbiota is in continuous interaction with the innermost layer of the gut, namely the epithelium. One of the various functions of the gut epithelium, is to keep the microbes at bay to avoid overstimulation of the underlying mucosa immune cells. To do so, the gut epithelia secrete a variety of antimicrobial peptides, such as chromogranin A (CgA) peptide catestatin (CST: $hCgA_{352-372}$). As a defense mechanism, gut microbes have evolved antimicrobial resistance mechanisms to counteract the killing effect of the secreted peptides. To this end, we treated wild-type mice and CST knockout (CST-KO) mice (where only the 63 nucleotides encoding CST have been deleted) with CST for 15 consecutive days. CST treatment was associated with a shift in the diversity and composition of the microbiota in the CST-KO mice. This effect was less prominent in WT mice. Levels of the microbiota-produced short-chain fatty acids, in particular, butyrate and acetate were significantly increased in CST-treated CST-KO mice but not the WT group. Both CST-treated CST-KO and WT mice showed a significant increase in microbiota-harboring phosphoethanolamine transferase-encoding genes, which facilitate their antimicrobial resistance. Finally, we show that CST was degraded by *Escherichia coli* via an omptin-protease and that the abundance of this gene was significantly higher in metagenomic datasets collected from patients with Crohn's disease but not with ulcerative colitis. Overall, this study illustrates how the endogenous antimicrobial peptide, CST, shapes the microbiota composition in the gut and primes further research to uncover the role of bacterial resistance to CST in disease states such as inflammatory bowel disease.

The multiple function of different phosphatidic acid species along the different steps of neuroendocrine secretion

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Chromaffin cells, as secretory cells, release hormones and neuropeptides for intercellular communication. This physiological function includes vesicular trafficking, from biogenesis of secretory granules from the Golgi cisternae, then transport towards the cell plasma membrane and finally release of the granular content by calcium-regulated exocytosis. This last process sequentially involves docking, priming and fusion of the granules to the exocytotic sites at the plasma membrane, and is followed by subsequent compensatory endocytosis to maintain cell membrane homeostasis. These trafficking events are regulated by membrane-associated proteins, but also lipids, as major components of membranes. However, little is known about contribution of the lipidic membrane composition in neuroendocrine secretion. Beyond the diversity of lipids, the tiny phospholipid phosphatidic acid (PA) appears as a key player.

Using adrenal chromaffin cells as a model, associated with genetic, silencing and pharmacological approaches, we report here that formation of PA from phosphatidylcholine by the enzyme phospholipase D1 (PLD1) regulates catecholamine release efficiency. Moreover, using a combination of electrochemical measurement of single cell catecholamine secretion and electron microscopy of roof-top membrane sheets, we have shown that diverse PA species, depending on their unsaturation degree, are involved in granule docking and fusion kinetics. Then we extended this work to discover that PA not only modulates calcium-regulated exocytosis, but also granular transport to the cell periphery and compensatory endocytosis. Indeed, using spinning disk confocal microscopy, we unraveled that inhibition of PLD activity also reduced the velocity of granules undergoing a directed motion under the plasma membrane. Furthermore, dopamine β-hydroxylase internalization assays revealed that PA production by PLD is required for an optimal recovery of vesicular membrane content by compensatory endocytosis. Thus, PA seems an important modulator of different steps underlying neuroendocrine secretion, supporting findings in other cell models. Now we need to address if these multiple functions are linked to the production of specific saturated, mono- or poly-unsaturated species of PA along the secretory pathway, which may provide key understanding of the physiological functions of these different forms of lipids.

Requirement of cholesterol for calcium-dependent vesicle fusion by stabilizing synaptotagmin-1-induced membrane bending

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Cholesterol is essential for neuronal activity and function. Cholesterol depletion in the plasma membrane impairs synaptic transmission. However, the molecular mechanisms by which cholesterol deficiency leads to defects in vesicle fusion remain poorly understood. Here we show that cholesterol is required for Ca^{2+}-dependent fusion using the *in-vitro* reconstitution of vesicle fusion, atomic force microscopy (AFM), and amperometry to monitor exocytosis in chromaffin cells. Purified native vesicles were crucial for the complete reconstitution of physiological Ca^{2+}-dependent fusion, whereas vesicle-mimicking liposomes failed to reproduce the cholesterol effect. Intriguingly, cholesterol had no effect on membrane insertion of synaptotagmin-1, a Ca^{2+} sensor for ultrafast fusion. Cholesterol stabilizes local membrane bending induced by synaptotagmin-1 insertion, thereby lowering the energy barrier for Ca^{2+}-dependent fusion to occur. Our data provide evidence that cholesterol depletion abolishes Ca^{2+}-dependent vesicle fusion by disrupting synaptotagmin-1-induced membrane bending, and suggests that cholesterol is a master regulator for Ca^{2+}-dependent fusion.

Membrane Fusion alterations caused by Sphingosine derivatives in Chromaffin cells.

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The fusion of membranes is a central part of the physiological processes involving the intracellular transport and maturation of vesicles and the final release of their contents, such as neuro-transmitters and hormones, by exocytosis. Traditionally, in this process, proteins, such SNAREs have been considered the essential components of the fusion molecular machinery, while lipids have been seen as merely structural elements. Nevertheless, sphingosine, an intracellular signalling lipid, greatly increases the release of neurotransmitters in neuronal and neuroendocrine cells, affecting the exocytotic fusion mode through the direct interaction with SNAREs. Moreover, recent studies suggest that FTY-720 (Fingolimod), a sphingosine structural analogue used in the treatment of multiple sclerosis, simulates sphingosine in the promotion of exocytosis. Furthermore, this drug also induces the intracellular fusion of organelles such as dense vesicles and mitochondria causing cell death in neuroendocrine cells. Therefore, the effect of sphingosine and synthetic derivatives on the heterologous and homologous fusion of organelles can be considered as a new mechanism of action of sphingolipids influencing important physiological processes, which could underlie therapeutic uses of sphingosine derived lipids in the treatment of neurodegenerative disorders and cancers of neuronal origin such neuroblastoma.

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Greasing the wheels of memory with myristic fatty acids

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Elucidating the mechanism by which phospholipases access neuronal membranes to process phospholipid substrates during neuroexocytosis and memory acquisition is critical to expanding our understanding of the complex bases for neurological diseases. We recently demonstrated that consolidation of long-term fear memory in rats is associated with a strong increase in saturated free fatty acids (FFAs; predominantly myristic, palmitic, and stearic acids). This suggests that members of the phospholipase A1 (PLA1) family may cleave saturated FFAs from the sn-1 position of phospholipids during memory consolidation. The correlation between PLA1 and neuronal function has been further demonstrated by mutations in DDHD2 isoform of PLA1, which are associated with several neurological disorders including autism, schizophrenia, intellectual disability, and neuromuscular disorders including hereditary spastic paraplegia.
In my talk, I will discuss the impact of genetic ablation of PLA1 in mice (DDHD2-/-) on progressive neuromotor and cognitive decline and the disruption of FFA responses to memory acquisition, using longitudinal behavioural and lipidomic analysis over 3-12 months. I will also reveal our search for synaptic protein binders that controls the localisation and activity of DDHD2. Improved understanding of DDHD2-regulated lipid pathways may offer novel insights into mechanisms and therapeutic strategies for neurodegenerative diseases.

Keywords: Neuroexocytosis, Lipids, Phospholipase A1; Free fatty acids; Myristic acid; Learning and memory

ubMunc13-2, synaptotagmin-7 and phorbolesters/diacylglycerol: a triad for dense-core vesicle priming.

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Munc13 proteins are priming factors for SNARE-dependent exocytosis, which are activated by diacylglycerol (DAG)-binding to their C1-domain. Several Munc13 paralogs exist, but their differential roles are not well understood. We studied the interdependence of phorbolesters (DAG mimics) with Munc13-1 and ubMunc13-2 in mouse adrenal chromaffin cells. Although expression of either Munc13-1 or ubMunc13-2 stimulated secretion, phorbolester was only stimulatory for secretion when ubMunc13-2 expression dominated, but inhibitory when Munc13-1 dominated. Accordingly, phorbolester stimulated secretion in wildtype cells, or cells overexpressing ubMunc13-2, but inhibited secretion in Munc13-2/Unc13b knockout (KO) cells or in cells overexpressing Munc13-1. Phorbolester was even more stimulatory in the Munc13-1/Unc13a KO, showing that endogenous Munc13-1 limits the effects of phorbolester. Imaging showed that ubMunc13-2, but not Munc13-1, traffics to the plasma membrane with a time-course matching Ca2+-dependent secretion, and trafficking is independent of synaptotagmin-7 (syt7). However, in the absence of syt7, phorbolester became inhibitory for both Munc13-1 and ubMunc13-2 driven secretion, indicating that stimulatory phorbolester x Munc13-2 interaction depends obligatorily on functional pairing with syt7. Overall, DAG/phorbolester, ubMunc13-2 and syt7 form a stimulatory triad for dense-core vesicle priming.

Roles of SphK1/S1P axis in synaptic vesicle recycling in neurons

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S1P is involved in the regulation of animal behaviors and may be linked to neurological disorders. Sphingosine kinase 1 (SphK1), the primary kinase for S1P production in mouse brain, is abundant within presynaptic terminals, indicating an importance of SphK1/S1P axis in presynaptic physiology. However, the mechanism by which SphK1/S1P regulating synaptic vesicle exocytosis and endocytosis remain unclear. By examining effects of a dominant negative SphK1 that disrupts S1P production, the present study demonstrates that SphK1/S1P axis may be important for synaptic vesicle exocytosis and endocytosis in neurons. The role of SphK1/S1P in synaptic vesicle exocytosis may need activations of S1P1/S1P3 receptors and TRPC5-mediated presynaptic Ca^{2+} signaling seems be critical for the role of SphK1/S1P axis in synaptic vesicle endocytosis. Collectively, our study points out distinct mechanisms for the role of SphK1/S1P axis in synaptic vesicle exocytosis and endocytosis in neurons.

Unique and shared principles in the secretion of chemical signals and their dysregulation in SNAREopathies

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The regulated secretion of chemical signals in different secretory cell types shares several features with older secretion principles, for instance in yeast, most notably the SNARE proteins that execute fusion and a SEC1p/Munc18-1 protein that organizes SNARE-complex assembly. However, in mammals, secretion principles have substantially diverged among different cell types and secretory organelles. My lab has studied unique and shared principles in docking, priming, Ca^{2+}-triggering and fusion of secretory vesicles in chromaffin cells and neurons and discovered distinct features in all of these steps using new photonic approaches to quantitatively characterize secretory vesicle fusion of many cargo types in living rodent and human secretory cells with single vesicle resolution. This presentation highlights specific arrangements to produce highly divergent secretion responses depending on different stimulation patterns, including different roles of the RAB3A/C/D-RIM1-Munc13 complex, different Ca^{2+}-channels and internal Ca^{2+}-stores, and different Ca^{2+}-sensors. Finally, mutations in all core components of the core secretion machinery have now firmly been implicated in human disease (SNAREopathies). This presentation will review the first insights into the underlying disease mechanisms and the remarkable diversity in clinical symptoms among SNAREopathy cases.

A secretory vesicle failure in Parkinson's disease occurs in human platelets

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Since platelets use similar, if not the same, mechanisms to accumulate serotonin (5-HT) as dopaminergic neurons to store DA in their VS; we wonder if a functional failure in the handling of 5-HT would reflect what is happening in dopaminergic neurons and in others of aminergic lineage. This could be diagnostic and prognostic platform.

The presence of high cytosolic concentrations of dopamine (DA) and its metabolites in neurons has been associated with increased vulnerability associated with Parkinson's disease (PD). More than 99% of the amines are confined to secretory vesicles (VS), making these structures crucial for keeping cytosolic DA low. Platelets have been used as cell models of various neurological diseases.

We have used freshly isolated blood platelets from 70 patients with PD, 113 control individuals and 21 patients with parkinsonism (iatrogenic origin, multi-systemic atrophy, dementia associated with Lewy bodies, progressive supranuclear palsy or parkinsonism of vascular origin). We have carried out a functional assay of 5-HT handling in human platelets in which its basal content and its capacity for accumulation, secretion and spontaneous loss have been quantified.

We found a drastic decrease in 5-HT content and uptake, as well as a decrease in thrombin-induced release in platelets from PD patients, but not in most cases of parkinsonism. Platelets from PD patients had impaired ability to retain 5-HT in SV.

These findings indicate a functional impairment of the SVs for amine handling in patients with PD. We will discuss its use of this technique as i) a biomarker, ii) its potential capacity for preclinical detection of PD and iii) how these tests can serve as a platform to screen disease-modifying drugs.

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Altered exocytosis and mitochondrial function in chromaffin cells from the SOD1^{G93A} mouse model of amyotrophic lateral sclerosis

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Alterations in cell excitability, ion channels, Ca2+ handling and exocytosis have been reported in chromaffin cells (CCs) of various transgenic mouse models of neurodegenerative diseases (de Diego et al., 2019; Carbone et al., 2019). In an early study in CCs from the SOD1G93A mouse model of amyotrophic lateral sclerosis, at disease stages when motor deficits were already established, we found with respect to wild type (WT) mice, a slower opening, expansion and closure of the exocytotic fusion pore; these changes were associated to pronounced reduction in cell excitability and ion currents driving action potentials (Calvo-Gallardo et al., 2015). More recently, we studied mitochondrial alterations from a structural, bioenergetic and functional perspective at early presymptomatic and symptomatic stages. The mitochondrial accumulation of the mutated SOD1G93A protein and the down-regulation of optic atrophy protein-1 (OPA1) provoke mitochondrial ultrastructure alterations prior to the onset of clinical symptoms. These changes affect mitochondrial fusion dynamics, triggering mitochondrial maturation impairment and cristae swelling, with increased size of cristae junctions. The functional consequences are a loss of mitochondrial membrane potential and changes in the bioenergetic profile, with reduced maximal respiration and spare respiratory capacity of mitochondria, as well as enhanced production of reactive oxygen species. This study identifies mitochondrial dynamic regulator OPA1 as an interesting therapeutic target in ALS. Additionally, our findings in the adrenal medulla gland from presymptomatic stages highlight the relevance of sympathetic impairment in this disease. Specifically, we show new SOD1G93A toxicity pathways affecting cellular energy metabolism in non-motor neurons, which offer a possible link between cell specific metabolic phenotype and the progression of ALS.

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Keywords: amyotrophic lateral sclerosis, SOD1G93A, chromaffin cell, mitochondrial dysfunction, OPA1

Electrochemical meaurements of temporal aspects of exocytotic plasticity in chromaffin and PC12 cell models

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In recent years, micro-/nano-electrochemical approaches have been developed to realize the quantitative measurement of intravesicular content and real-time monitoring of their release dynamics. We have used vesicle impact electrochemical cytometry (VIEC), and subsequently stochastic VIEC and intracellular VIEC (IVIEC) to provide a highly effective way to quantify the electroactive contents inside vesicles. This has allowed direct comparison of the quantity of molecules released by exocytosis to those in the vesicles. Partial release is observed across all cell types examined to date by the electrochemical methods and appears to be regulated. We have confirmed this with NanoSIMS imaging of dopamine lost and external drug captured by closing vesicles in stimulated PC12 cells.
We have used electrochemistry and imaging mass spectrometry with static SIMS to directly examine exocytotic plasticity in PC12 cells as a model. A new electrochemical approach with both intracellular and extracellular measurements at the same time has led to time resolved measurements of plasticity in real time. This plasticity is apparent as a change in fraction released which is observed in drug-treated cells and by decreasing the fraction of vesicular release in response to repetitive stimulation iron deficiency has a negative effect on plasticity.
Finally, in a new technical approach, we have discovered that the chaotropic anion (SCN^-) creates a two-step process (around 30% doublet peaks) when examining adrenal chromaffin vesicles with VIEC. We have then used this to independently count molecules in each subvesicular compartment, the halo and protein dense-core, and shown the fraction of catecholamine binding to the dense-core is 68%. Moreover, we differentiated two distinct populations of large dense-core vesicles (LDCVs) and quantified their content, which might correspond to immature (43%) and mature (30%) LDCVs, to reveal differences in their biogenesis. We speculate this is caused by an increase in the electrostatic attraction between protonated catecholamine and the negatively charged dense-core following adsorption of SCN^- anion.

Visualizing membrane dynamics of exo- and endocytosis

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Membrane dynamics of exo- and endocytosis have been speculated based on electron microscopic observations. Here I will describe the recent progresses from my lab in the study of membrane dynamics of exo- and endocytosis in bovine adrenal chromaffin cells. With super-resolution STED microscopy, we have directly visualized the entire fusion pore dynamic process, including hemi-fusion, hemi-to-full fusion, fusion pore opening and expansion, fusion pore constriction and closure. We visualized various fusion modes involving fused vesicle size change, including shrink-fusion, same-size fusion, enlarge-fusion. We also visualized sequential compound fusion in live cells. For endocytosis, we visualized the flat to round transformation in live cells for the first time. I will also describe our progresses in understanding the mechanisms underlying the above-mentioned exo- and endocytosis membrane dynamics.

Vesicle docking: Mechanical interactions between SNARE proteins revealed by atomic force spectroscopy

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Interactive mechanical forces between pairs of individual SNARE proteins synaptobrevin 2 (Sb2) and syntaxin 1A (Sx1A) may be sufficient to mediate vesicle docking. This notion, based on force spectroscopy single molecule measurements probing recombinant Sx1A an Sb2 *in silico,* questioned a predominant view of docking via the ternary SNARE complex formation, which includes an assembly of the intermediate *cis* binary complex between Sx1A and SNAP25 on the plasma membrane to engage Sb2 on the vesicle. However, whether a *trans* binary Sx1A-Sb2 complex alone could mediate vesicle docking in a cellular environment remains unclear. To address this issue, we used atomic force microscopy (AFM) in the force spectroscopy mode combined with fluorescence imaging. Using AFM tips functionalized with the full Sx1A cytosolic domain, we probed native Sb2 studding the membrane of secretory vesicles docked at the plasma membrane patches, referred to as “inside-out lawns”, identified based on fluorescence stains and prepared from primary culture of lactotrophs. We recorded single molecule Sx1A-Sb2 mechanical interactions and obtained measurements of force (~183 pN) and extension (~21.6 nm) necessary to take apart Sx1A-Sb2 binding interactions formed at tip-vesicle contact. Measured single interactive force between a pair of Sx1A-Sb2 molecules is sufficient to hold a single secretory vesicle docked at the plasma membrane within distances up to that of the measured extension. This finding further advances a notion that native vesicle docking can be mediated by a single *trans* binary Sx1A-Sb2 complex in the absence of SNAP25.

Exocytotic release of surfactant from the alveolar type II lung cells in health and disease

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Secretion of pulmonary surfactant in the alveoli is essential to maintain lung function. Pulmonary surfactant is secreted via exocytosis of lamellar bodies (LBs), large lysosome-related storage organelles in alveolar type II (ATII) epithelial cells. Modest elevations of the intracellular Ca^{2+} concentration are sufficient to induce LB fusions with the plasma membrane. However, surfactant is a poorly soluble, lipoprotein-like substance that is not readily released from fused LBs following opening of the fusion pore. Efficient release requires an expansion of the fusion pore and active extrusion mechanisms during the exocytic post-fusion phase. Fusion pore expansion depends on a localized, fusion-activated Ca^{2+} elevation (FACE) around fused LBs. FACE is triggered by the rise in intra-vesicular pH following fusion pore opening and activation of $P2X_4$ receptors on the LB membrane by ATP stored in LBs. Subsequently, Ca^{2+} binds to synaptotagmin-7 on fused LBs to antagonize the recruitment of complexin-2 and facilitates fusion pore expansion. In addition, LBs are selectively coated with actin following fusion with the PM. Compression of this actin coat provides the physical force required for surfactant expulsion from LBs. This depends on myosin motor activity and regulated actin depolymerization and crosslinking. The (patho)physiological implications of this elaborate post-fusion exocytic machinery are still elusive. Mechanical squeezing of surfactant through the fusion pore could help in the transformation and activation of the tightly packed macromolecular surfactant assembly stored in LBs. In addition, recent evidence also suggested a link between the exocytic release and surfactant activation via FACE. FACE drives apical (luminal) fluid resorption in the alveolus and facilitates adsorption of newly released surfactant into the air–liquid interface. A mechanism likely disturbed in alveolar edema situations.

Fusion pore stabilization by cholesterol in astrocytes and pituitary cell; relevance for lysosomal storage disease

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Lysosomal storage diseases (LSDs) are a group of heritable metabolic disorders, characterized by the accumulation of undigested large molecules, including cholesterol, in lysosomes. Cholesterol is an essential component of the cellular membranes and plays a role in membrane fusion. We asked, whether increased vesicle cholesterol affects vesicle fusion with the plasmalemma in two physiologically distinct cell types, lactotrophs and astrocytes, which both exhibit Ca^{2+}-dependent exocytosis, however, regulated by distinct Ca^{2+} sources. We show that cholesterol depletion enhances cytosolic levels of Ca^{2+} in lactotrophs but decreases it in astrocytes. Nevertheless, cholesterol depletion evoked vesicle secretion in lactotrophs as well as in astrocytes. Super-resolution microscopy revealed that in lactotrophs regulated exocytosis promotes cholesterol delivery to the plasmalemma. To determine, if cholesterol directly defines the fusion pore, a channel-like structure between the vesicle lumen and the extracellular space, we have performed high-resolution membrane capacitance measurements. These measurements, which were used to monitor fusion pore conductance, confirmed that extraction of cholesterol increases, while enrichment with cholesterol decreases the conductance of the fusion pore. To assess if the fusion pore can be modified in diseases, we repeated electrophysiologocal measurements in a cellular model of LSD - mouse fibroblast lacking the Niemann-Pick Disease Type C1 (Npc1) protein, in which cholesterol accumulates in vesicles. Results from these model confirmed previous observations, as the fusion pore conductance was smaller than in controls, showing that vesicle cholesterol controls fusion pore and is relevant for pathophysiology of LSD. In an attempt to explain this mechanism, a new model of cholesterol-dependent fusion pore regulation is proposed.

Heterologous lysosomal fusion and the cell-based immunotherapy of prostate cancer

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Antigen presentation is based on lysosomal fusion with the plasmalemma, allowing the antigens complexed with major histocompatibility molecules II (MHC II), resident in lysosomes, to be presented on the surface of the cell, engaging their recognition by neighboring and immune cells. The nature of lysosomal interaction with the plasmalemma was studied by using high-resolution membrane capacitance measurements in reactive astrocytes, abundant neuroglial cells in the central nervous system, in which vesicle dynamics is remodeled in pathological conditions. To generate heterologous lysosomal fusion constructs, we electrofused tumor and dendritic, antigen presenting, cells, to generate hybridomas carrying tumor antigens and the machinery of dendritic cells necessary for antigen presentation. The former experiments revealed that in induced reactive astrocytes, which acquire the mechanism(s) of antigen presentation, the abundance of lysosomal exocytotic interactions with the plasmalemma increased with a prolonged transient open fusion pore dwell time and reduced endocytosis[1] . To generate artificial imunohybridoma cells, with fused heterologous lysosomes[2] and with immunostimulatory capacity[3] to be used in cellular immunotherapy in patients with castration-resistant prostate cancer (CRPC). In a double-blind, placebo-controlled, cross-over clinical trial CRPC patients were treated with the autologous immunohybridoma (aHyC) vaccine by intradermal injection, revealing that this complex approach is feasible, non-toxic, preserves quality of life and modulates immune response by reducing the $CD56^{bright}CD16^{-}$ natural killer cells in the peripheral blood. Patient survival was inversely correlated with changes in $CD56^{bright}CD16^{-}$ natural killer cells, consistent with reports of their pro-metastatic role. In conclusion, aHyC, a completely personalized cell-based vaccine, treats CRPC patients and has potential for use in the treatment of other solid tumors[4, 5].

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Dynamic organization of voltage gated calcium channels in the presynaptic membrane

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Voltage gated calcium channels (VGCC) are essential for the conversion of electrical in chemical signals, which has been well investigated within chemical synapses. The precision and reliability of synaptic information transfer is critical dependent on the number, kinetic properties and the localization of VGCC within the presynaptic membrane. We used single molecule tracking to access the number and localization of individual $Ca_V2.1$-channels over time in synapses of hippocampal mouse neurons. Either, overexpression of the pore forming subunit of $Ca_V2.1$-channels or tracking of the endogenous population of channels confirmed that these channels are confined but dynamic organized within the presynaptic membrane. It has been shown that particular interactions between the C-terminus of the pore forming channel subunit and scaffold proteins are essential to localize $Ca_V2.1$-channels in the pre-synapse. Using different splice variants that either alter the interaction of $Ca_V2.1$-channels with scaffold proteins or influence their calcium sensitivity let conclude that the attraction of the channels to the presynaptic membrane depend partially on the configuration of the C-terminus. Furthermore, the release probability of the synapse can be influenced by the predominant splice variant and their dynamic organization within the membrane. Using light induced cross-linking induces transient changes in the release probability of the synapse, supporting a mobile arrangement of $Ca_V2.1$-channels within the presynaptic membrane.
Apart from the confined local organization inside the synapse we realized that a substantial fraction of $Ca_V2.1$-channels is also transported between synapses in intracellular vesicles. After bleaching the synaptic population of channels there is a rapid reoccurrence of confined channels within the synapse suggesting an unexpected fast replacement/turnover of $Ca_V2.1$-channels. To which extend a vesicular channel population links to a fast change in the release properties of the synapse remain to be determined.

Chromaffin cell firing viewed through MEAs: evidence for two distinct firing modes and well-resolved action potential-driven Na^+, K^+ and Ca^{2+} currents at the cell-microelectrode contact region

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Rat chromaffin cells (CCs) cultured on MEAs for 1-2 days fire spontaneously when placed on a recording system at 37 °C. CCs on MEAs secrete also catecholamines when tested with CFE in 2 mM [Ca^{2+}] during muscarinic stimulation.
Extracellularly recorded APs (EAPs) occur in two opposing forms: as dominant downward (*negative going*) or as dominant upward deflections (*positive going*) with two distinct modes of firing. One mode is "continuous" with 4.3 Hz mean frequency at 37 °C, which is 2.4 faster than the frequency of intracellularly recorded APs (IAPs), at 22 °C. This firing occurs less often (~30% of MEAs) with respect to the second firing mode (~70% of MEAs). This latter occurs as high frequency "intermittent bursts" of 7 s duration separated by silent periods of 12 s. AP frequency during bursts is normally distributed around 7.9 Hz (n= 44), regardless of the direction of EAPs. This "intermittent firing" is rarely observed in IAPs recorded at 22 °C from rat CCs on plastic dishes.
Most of the *positive going* EAPs resemble the classical IAPs and appear mainly in the intermittent mode. *Negative going* EAPs occur more frequently in both the continuous and intermittent modes. They have a transient inward component of 40-100 μV amplitude with half-width of ~0.33 ms, followed by a transient outward component of 10-20 μV, lasting 3-5 ms. Following the common interpretation of EAPs from neurons (Schatzthauer & Fromherz, 1998), *negative going* EAPs represents the trajectory of the inward Na^+/Ca^{2+} and outward K^+ currents passing through the membrane portion of CCs in tight contact with the MEAs during an AP. In agreement with this, the inward Na^+ current component of EAPs is blocked by TTX in a dose-dependent manner (IC_{50} ~10 nM) while the outward component is almost fully blocked by the BK channel blocker paxilline (200 nM) or 5 mM TEA. The SK channel blocker apamin (200 nM) has no effect on EAPs. In a minority of cells, expressing low densities of K channels, inward Na^+ and Ca^{2+} currents are evident after full block of BK channels. Block of BK channels causes also a reduction of AP frequency regardless of the firing mode.
In conclusion, MEA recordings furnish an effective way to record the dual AP firings of rat CCs with direct access to the Na^+, K^+ and Ca^{2+} currents that sustain the firing.

Loss of $\alpha_2\delta$-1 calcium channel subunit function increases excitability and promotes burst firing in mouse chromaffin cells

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High voltage gated calcium channels (HVCC) shape the electrical activity and control hormone release in most endocrine cells. HVCCs are multi-subunit protein complexes formed by the pore forming α1 and the auxiliary β, α2δ and γ subunits. Here we show that α2δ-1 deletion, the highest expressed α2δ isoform in mouse chromaffin cells (MCCs), led to ~60% reduced HVCC calcium influx with slower inactivation kinetics. Pharmacological dissection showed that HVCC composition remained similar in α2δ-$1^{-/-}$ MCCs compared to WT demonstrating that α2δ-1 exerts similar functional effects on all HVCC isoforms. Consistent with reduced HVCC calcium influx the action potentials (AP) in α2δ-$1^{-/-}$ MCCs showed a lower peak amplitude, and faster rising and decay slopes resulting in shorter half-maximal AP duration. Additionally, the number of cells showing spontaneous activity was significantly reduced upon α2δ-1 deletion. However, the induced electrical activity showed a significantly higher frequency in α2δ-$1^{-/-}$ MCCs as well as an increase in the number of cells with burst firing compared to WT that preferentially display tonic firing. Additionally, despite the reduced HVCC calcium influx, the readily releasable pool and total vesicle exocytosis and endocytosis following a 500ms calcium preloading step were unaltered in α2δ-$1^{-/-}$ compared to WT MCCs. The similar exocytosis in α2δ-$1^{-/-}$ MCCs compared to WT was not caused by a compensatory upregulation of intracellular calcium release. In conclusion, our study suggests that α2δ-1 genetic deletion leads to increased catecholamine secretion due to increased excitability and preserved vesicle exocytosis despite a lower calcium influx.
Acknowledgements: (FWF) P31434 and DOC30-B30 to PT and F18863 to SMG.

CLC anion/proton exchangers regulate secretory vesicle filling and granule exocytosis in chromaffin cells

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ClC-3, ClC-4, and ClC-5 are electrogenic chloride/proton exchangers that can be found in endosomal compartments of mammalian cells. Although the association with genetic diseases and the severe phenotype of knockout animals illustrate their physiological importance, the cellular functions of these proteins have remained insufficiently understood. We here study the role of two *Clcn3* splice variants, ClC-3b and ClC-3c, in granular exocytosis and catecholamine accumulation of adrenal chromaffin cells using a combination of high-resolution capacitance measurements, amperometry, protein expression/gene knock-out/down, rescue experiments, and confocal microscopy. We demonstrate that ClC-3c resides in immature as well as in mature secretory granules, where it regulates catecholamine accumulation and contributes to the establishment of the readily releasable pool of secretory vesicles. The lysosomal splice variant ClC-3b contributes to vesicle priming only with low efficiency and leaves the vesicular catecholamine content unaltered. The related Cl^-/H^+ antiporter ClC-5 undergoes age-dependent down-regulation in wild-type conditions. Its upregulation in *Clcn3*$^{-/-}$ cells partially rescues the exocytotic mutant defect. Our study demonstrates how different CLC transporters with similar transport functions, but distinct localizations can contribute to vesicle functions in the regulated secretory pathway of granule secretion in chromaffin cells.

High Performance Photomanipulation

Martin Brill & Gert Rapp

Rapp OptoElectronic GmbH, Kronskamp 110, 22880 Wedel, Germany

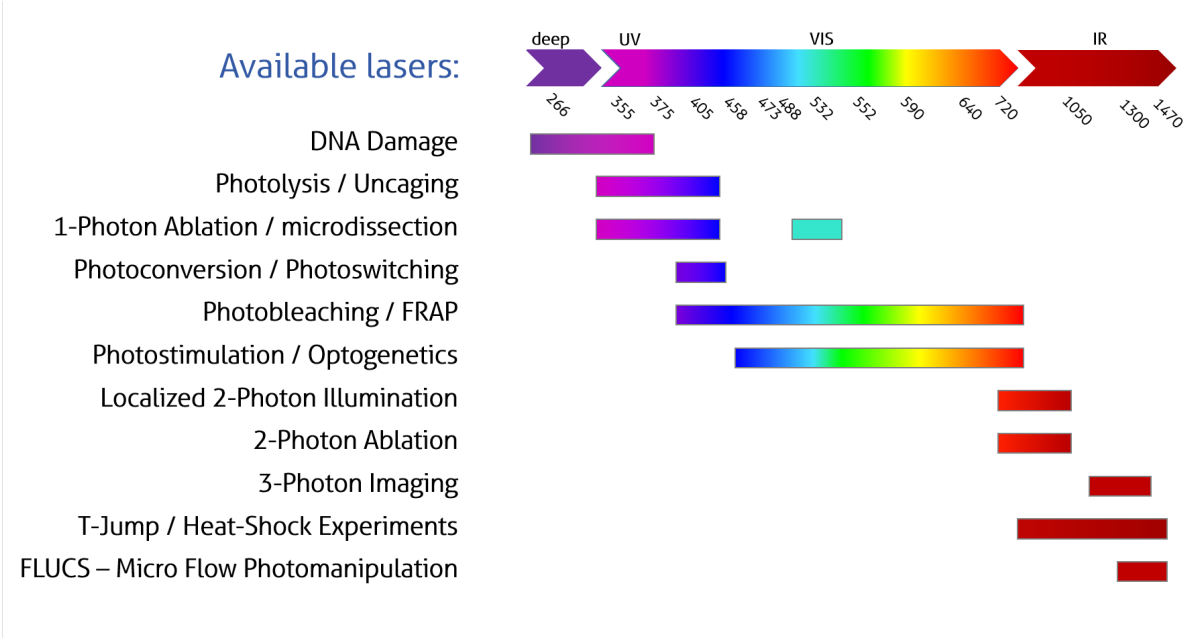
The understanding of living organisms requires structural and functional knowledge of their parts and their respective interplay. Having tools to manipulate, perturb or activate these parts allows in-depth understanding of the organism.

Using light to release ions like Ca^{2+}, nucleotides (e.g. ATP) or neurotransmitters (e.g. glutamate) from photo-labile precursors has helped tremendously understanding cellular processes. Decades ago we started with uncaging devices like flash lamp systems for temporally and spatially precise release of such compounds.

With the development of scanner-based laser systems we increased the range of applications. Amongst them are experiments using FRAP for understanding of intracellular transport processes. Further, equipment for optogenetic stimulation, UV-DNA-damage with dedicated deep UV microscope modifications, photoconversion or IR-temperature jump applications and time-resolved electron microscopy.

Latest activities are 2- and 3-photon microscopy with dedicated 2-photon SLM based photo-stimulation as well as FLUCS – focused light induced cytoplasmatic streaming – a new technique for micro flow induced photo-manipulation in living cells.

A summary of applications covered by our devices is given in the figure below.



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Tools for Patch Clamp, Imaging and Capacitance Measurements

Martin Oberhofer

HEKA Elektronik, Reutlingen, Germany

HEKA provides high-quality software and hardware products for electrophysiological and electrochemical research applications. In this presentation we want to highlight some tools for Imaging and Capacitance measurements. Further, there will be a live presentation of the new Patchmaster Next software showing some capacitance measurements features.

VISITIRF ORBITAL-RINGTIRF TECHNOLOGY

VISIVIEW 6.0 - IMAGING SOFTWARE WITH NEW REALTIME 5D IMAGE ACQUISITION AND DISPLAY

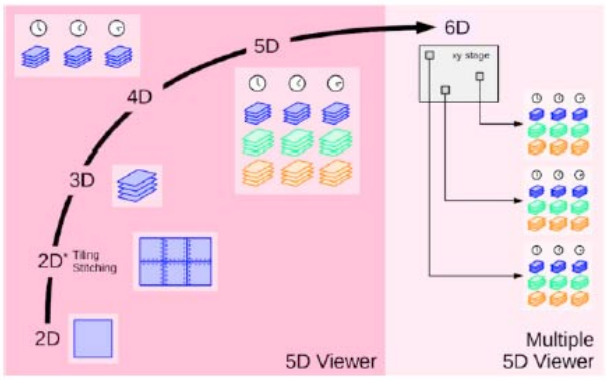
Dr. Steven Bump
Visitron Systems GmbH, Benzstraße 36, D-82178 Puchheim, Germany,
sbump@visitron.de

KEY WORDS: Living cells microscopy with RingTIRF, news in Microscopy Imaging Software with AI

1. AIMS This workshop will give the listener an overview of new developments in RingTIRF technology and new features in VisiView Imaging Software

2. ORBITAL Ring-TIRF Technology The VisiTIRF-ORBITAL is a compact and powerful high speed 2D galvo driven spinning Ring-TIRF laser illumination system. It offers a large and evenly illuminated field of view to enable applications such as single molecule tracking or SMLM - Single Molecule Localisation Microscopy for superresolution imaging. Full 360 degree positioning by free circular diameter or elliptical trajectory at the back focal plane of the high aperture TIRF objective offers illumination with minimal fringes or shading gradients

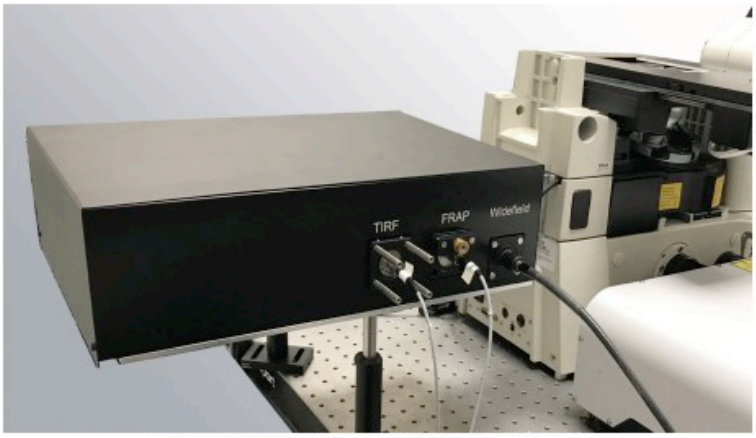
2. VisiView Microscopy Imaging Software The workflow oriented VisiView design offers an intuitive imaging platform for most complex multidimensional life science application. The flexible and individual hardware support of any microscope platform, scientific grade camera or high quality microscopy peripheral, including Confocal, FRAP or TIRF control offers each researcher the optimum solution. If a certain device is missing, we as a product independent manufacturer, are very quick in implementation.



6D Experiments available with VisiView and VisiScope 4Elements:

2D	Single camera field-of-view
2D*	Multiple adjacent camera field-of-view
3D	Z-Stack
4D	Time Series
5D	Multi-Channel
6D	Non-adjacent xy stage positions

Figure 1: VisiView Imaging Software



ORBITAL-500 on Nikon Eclipse Ti microscope

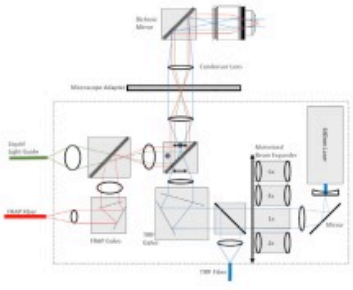


Figure 2: Orbital RingTIRF Scanner

Vascular contribution in Alzheimer's disease associated blood brain barrier damage, neuroinflammation and cognitive decline

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Alzheimer's disease (AD) is the most common neurodegenerative disease, affecting millions of people worldwide. Extracellular beta-amyloid (Aβ) plaques and neurofibrillary tau tangles are classical hallmarks of AD pathology and thus are the prime targets for AD therapeutics. However, approaches to slow or stop AD progression and dementia by reducing Aβ production, neutralizing toxic Aβ aggregates, or inhibiting tau aggregation have been largely unsuccessful in clinical trials. The contribution of dysregulated vascular components and inflammation is evident in AD pathology. Vascular changes are detectable early in AD progression, so treatment of vascular defects along with anti-Aβ/tau therapy could be a successful combination therapeutic strategy for this disease. The present work will address how vascular dysfunction mechanistically contributes to neuroinflammation, neurodegeneration, and cognitive decline in AD. This work will also strengthen the idea that treating vascular dysregulation in AD could also improve brain function and memory along with minimizing vascular defects.

Functional Determination of Calcium Binding Sites Required for the Activation of Inositol 1,4,5-trisphosphate receptors

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Inositol 1,4,5-trisphosphate (IP_3) receptors (IP_3Rs) initiate a diverse array of physiological responses by carefully orchestrating intracellular calcium (Ca^{2+}) signals in response to various external cues. Notably, IP_3R channel activity is determined by several obligatory factors including IP_3, Ca^{2+} and ATP. The critical basic amino acid residues in the N-terminal IP_3-binding core (IBC) region that facilitate IP_3 binding are well characterized. In contrast, the residues conferring the biphasic regulation by Ca^{2+} are yet to be ascertained. Using comparative structural analysis of Ca^{2+} binding sites identified in two main families of intracellular Ca^{2+}-release channels, ryanodine receptors (RyRs) and IP_3Rs, we identified putative acidic residues coordinating Ca^{2+} in the cytosolic calcium sensor region in IP_3Rs. We determined the consequences of substituting putative Ca^{2+} binding, acidic residues in IP_3R family members. We show that the agonist-induced Ca^{2+} release, single channel open probability (P_0) and Ca^{2+} sensitivities are markedly altered when the negative charge on the conserved acidic side chain residues are neutralized. Remarkably, neutralizing the negatively charged side chain on two of the residues individually in the putative Ca^{2+} binding pocket shifted the Ca^{2+} required to activate IP_3R to higher concentrations, indicating that these residues likely are a component of the Ca^{2+} activation site in IP_3R. Taken together, our findings indicate that Ca^{2+} binding to a well conserved activation site is a common underlying mechanism resulted in increased channel activity shared by IP_3Rs and RyRs.

A CRIg+ macrophage-dependent defense against microbial extracellular vesicle-induced adrenomedullary dysfunctions

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Objective: Obesity is an established risk factor for hypertension. Although obesity-induced gut barrier breach leads to the leakage of various microbiota-derived products into host circulation and distal organs, roles of microbiota in mediating the development of obesity-associated adrenomedullary disorders and hypertension have not been elucidated. We seek to explore impacts of microbial DNA enrichment on inducing obesity-related adrenomedullary abnormalities and hypertension.

Approach and Results: Obesity was accompanied by remarkable bacterial DNA accumulation and elevated inflammation in adrenal glands. Gut microbial DNA containing extracellular vesicles (mEVs) were readily leaked into bloodstream and infiltrated into adrenal glands in obese mice, causing microbial DNA enrichment. In lean WT mice, adrenal macrophages expressed CRIg that efficiently blocks the infiltration of gut mEVs. In contrast, adrenal CRIg+ cell population was greatly decreased in obese mice. In lean $CRIg^{-/-}$ or $C3^{-/-}$ mice intravenously injected with gut mEVs, adrenal microbial DNA accumulation elevated adrenal inflammation and norepinephrine secretion, concomitant with hypertension. In addition, microbial DNAs promoted inflammatory responses and norepinephrine production in PC12 cells treated with gut mEVs. Depletion of microbial DNA cargos markedly blunted the effects of gut mEVs. We also validated that activation of cGAS/STING signaling is required for the ability of microbial DNAs to trigger adrenomedullary dysfunctions in both *in vivo* and *in vitro* experiments. Restoring CRIg+ cells in obese mice decreased microbial DNA abundance, inflammation, and hypertension.
Conclusions: The leakage of gut mEVs leads to adrenal enrichment of microbial DNAs that are pathogenic to induce obesity-associated adrenomedullary abnormalities and hypertension. Recovering CRIg+ macrophage population attenuate obesity-induced adrenomedullary disorders.

The interplay between α7 nicotinic acetylcholine receptors, Pannexin-1 channels and P2X7 receptors regulate exocytosis in chromaffin cells

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Pannexin-1(Panx1), the most widely expressed pannexin family member, is a plasma membrane glycoprotein that forms channels permeable to signaling molecules such as ATP. A preliminary study by our group suggests a functional coupling between Panx1 channels and nicotinic acetylcholine receptors (nAChR), however the involvement of a specific nAChR such as the α7 nAChR has not been studied. In our present work we propose a new mechanism for Panx1 channel activation through a functional crosstalk with the highly Ca^{2+} permeable α7 nicotinic acetylcholine receptor (nAChR). We found that activation of α7 nAChRs induces Panx1-mediated dye uptake and ATP release in the neuroblastoma cell line SH-SY5Y-α7. This Panx1 channel opening was dependent on Ca^{2+} signals localized in submembrane areas, as well as on Src kinases. In turn, Panx1 channels amplify cytosolic Ca^{2+} signals induced by the activation of α7 nAChRs, by a mechanism that seems to involve ATP release and P2X7 receptor activation. The physiological relevance of this crosstalk was demonstrated in chromaffin cells, where Panx1 channels and P2X7 receptors contribute to the exocytotic release of catecholamines triggered by α7 nAChRs. We have furthermore determined that ATP co-released during Ca^{2+} dependent exocytosis affects, in an autocrine manner, secretion induced by a diverse set of secretagogues in chromaffin cells through activation of P2X7 receptors. This finding was corroborated using P2X7 KO mice wherein genetic deficiency of the channel negatively altered secretion. Our findings demonstrate (1) the existence of a functional coupling between α7 nAChRs, Panx1 channels and P2X7 receptors with physiological relevance in neurosecretion, and (2) the importance of P2X7 receptor as a mechanism to control exocytosis in chromaffin cells.

This work has been supported by the grants FONDECYT 3180140 to MCM, FONDECYT 1160495 to A.M.C, and P09-022-F from ICM-ECONOMIA to CINV.

A new predictive sensor for the doxorubicin-dependent cardiotoxicity: the paradigm of the cardioprotector chromogranin-A

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[1] Lab of Cellular and Molecular Cardiovascular Pathophysiology (Operative Unite of the National Institute of Cardiovascular Research), Department of Biology, E. and E.S., University of Calabria, Rende (CS), Italy; [2]Department of Health Science, University Magna Graecia of Catanzaro, 88100 Catanzaro, Italy; [3]San Raffaele Vita-Salute University and IRCCS San Raffaele Scientific Institute, Division of Experimental Oncology, Milan, Italy; [4]National Institute of Cardiovascular Research (INRC), Bologna, Italy.

Background The dose-dependent cardiotoxicity induced by the widely used anti-cancer chemotherapeutic drug Doxorubicin (DOX) critically hampers its therapeutic use. Therefore, several adjuvant therapies able to decrease the cardiotoxic effects of DOX are under continuous investigation. Because of the well-established cardioactive and cardioprotective effects of the multifunctional protein Chromogranin A (CgA), this study was undertaken to explore its ability to mitigate the DOX-dependent adverse cardiotoxic effects and regulate the antitumor activity of this anticancer drug.

Methods The effect of physio-pharmacological doses of full-length recombinant CgA was tested in *in vivo* and *ex vivo* rat models, and murine models of melanoma, fibrosarcoma, lymphoma and lung cancer. After DOX treatment, CgA plasma levels were evaluated in these murine models and in cancer patients.

Results *In vivo* protocols revealed that CgA is able to prevent Doxo-dependent systemic inflammation, myocardial fibrosis and cardiac damage, as visible by a reduction of IL-1β, TNF-α, ROS, LDH, and cTnT plasma levels together with a decrease of cardiac CTGF expression. Interestingly, the intra-cardiac expression of CgA (i.e., an important cardioprotective agent) and its release in the blood is reduced after DOX exposure. Also in patients with breast cancer and lymphoma, CgA progressively decreases upon treatment, suggesting that prolonged treatment with chemotherapy decreases the levels of these cardioprotective factor. On the other hand, CgA does not impair the anticancer DOX activity in tumour-baring mice models of melanoma, lymphoma, fibrosarcoma, and Lewis lung carcinoma. *Ex vivo* experiments showed that CgA reduces DOX-dependent cardiac ischemic susceptibility and its related inflammation by activating prosurvival pathways, i.e. RISK and SAFE cascades, and by decreasing NLRP3 expression and ROS production. Moreover, CgA protects the heart by turning off apoptosis as demonstrated by an increase of Bcl-2 and a decrease of Bax and active Caspase-3 in ventricular cardiomyocytes. Interestingly, the CgA-derived peptides CTS and VS1 also exert cardioprotective effects against DOX -induced cardiotoxicity in the rat model. However, in this case, cardioprotection could be achieved with 33 nmol/kg/d of peptide (i.e., with a dose much higher than that required for full-length CgA). Thus, full-length CgA is a more potent cardioprotective agent than its fragments.

Conclusions The detection of circulating CgA levels before, during and after chemotherapy can predict DOX-induced cardiotoxicity; moreover, the administration of exogenous CgA to cancer patients with low CgA plasma levels could be used as a support therapy to limit or prevent chemotherapy severe side effects without impairing its anti-tumoral efficacy.

Negative regulation of exocytosis by amisyn, the vertebrate-specific competitor of VAMP2

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Exocytosis, a process of vital importance for (neuro)endocrine and neuronal cells, is controlled by a set of both positive and negative regulators. While promotors of exocytosis are well studied, negative regulators are poorly understood. In parallel with the characterization of the proteinaceous machinery, the lipid requirements for SNARE-mediated vesicle exocytosis arose. Lipids are the core components of fusing membranes, thus changes in their composition, abundance and/or localization promptly modify the intrinsic fusogenic properties of membranes. Lipids also activate and recruit proteins to the local environments where exocytosis happens. We have previously reported a key lipid involved in spatial and temporal regulation of exocytosis – phosphatidylinositol-4,5-bisphosphate [$PI(4,5)P_2$]. We have now discovered that $PI(4,5)P_2$ engages a small SNARE protein, amisyn (STXBP6), to negatively regulate exocytosis. Amisyn acts as a vertebrate-specific competitor of synaptobrevin-2/VAMP-2, an essential player in exocytosis (PMID: 32467162). It is a poorly studied protein despite several studies reporting the occurrence of amisyn mutations in diabetes, autism and cancer. Amisyn contains a pleckstrin homology domain that mediates its transient association with the plasma membrane by binding to phospholipid $PI(4,5)P_2$. The characterization of amisyn functions in exocytosis, neurotransmission and behavior using cellular and knock-out mammalian models will be discussed.

Functional human variants of catestatin and pancreastatin peptides enhance cardiometabolic disease risk

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Chromogranin A (CHGA) is a ~50 kDa soluble and acidic glycoprotein that occurs abundantly in dense-core secretory vesicles of endocrine, neuronal and neuroendocrine cells. It was known as a marker for neuroendocrine tumors for several decades. However, studies by us and others in the recent years provided evidence for its role in cardiovascular homoeostasis. At the intracellular level, CHGA plays a key role in the biogenesis of the secretory vesicles. At the extracellular level, CHGA acts as a pro-hormone that generates bioactive peptides including the dysglycemic peptide pancreastatin (PST: $CHGA_{250-301}$) and anti-hypertensive/cardioprotective peptide catestatin (CST: $CHGA_{352-372}$). We probed for functional genetic variations in CST and PST domains in human populations. Among the CST variants Gly364Ser enhances the risk for hypertension (by ~1.5-fold) in Indian and Japanese populations. The increased disease risk associated with the 364Ser variant may be accounted for by diminished interactions of the CST-364Ser peptide with adrenergic beta-2 receptor leading to lower endothelial nitric oxide level as compared to the CST-364Gly wild-type peptide. More recently, we have found that PST Gly297Ser variant confers an increased risk (~1.3-1.6-fold) for type-2 diabetes/hypertension/coronary artery disease/metabolic syndrome in independent human populations. In corroboration, the variant peptide (PST-297Ser) displays gain-of-potency in several cellular events relevant for cardiometabolic disorders (*e.g.*, increased expression of gluconeogenic genes, increased catecholamine secretion, greater inhibition of insulin-stimulated glucose-uptake) than the wild-type peptide (PST-WT). Computational analysis and in vitro receptor-peptide binding assays show higher affinity binding of PST-297Ser peptide with glucose-regulated protein 78 (GRP78) and insulin receptor (IR) than PST-WT, providing a mechanistic basis for the enhanced activity of the variant peptide. Thus, CST-364Ser and PST-297Ser variants emerge as new risk factors for cardiometabolic disease.

The role of chromogranin A and catestatin in homeostasis and disease

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Introduction In a large population-based metagenomics study [Zhernakova, et al. (2006) Science 352:565], fecal levels of Chromogranin A, a neuroendocrine prohormone, were surprisingly found to strongly correlate with the gut microbiota composition. In fact, Chromogranin A was found to be more than 5-fold stronger associated than any other factor assessed, including diet (carbohydrates, alcohol, etc), medicine (antibiotics, PPI), smoking, and intrinsic factors (age, sex, cytokines). Moreover, blood levels of Chromogranin A have been associated with various inflammatory and metabolic pathological conditions, including inflammatory bowel disease (IBD), hypertension, and diabetes, indicating that this prohormone plays crucial roles in metabolism and the immune system. The pro-hormone chromogranin A (CgA) is secreted by endocrine organs including pancreas and enterochromaffin cells located in the gut. CgA is proteolytically cleaved intracellularly as well as extracellularly upon release, giving rise to several bioactive peptides that exert a wide variety of regulatory functions. In my talk, I will discuss how CgA and its cleavage product catestatin modulate health and disease via the regulation of immune system activity and the gut barrier function.

Method CgA and CST levels were measured in EDTA-plasma using ELISA and visualized in the colon of IBD patients by immunohistochemistry. Additionally, we generated mice with selective deletion of the CST-coding region of the *ChgA* gene (CST-KO). To study the colon in more detail we performed gut barrier function assays. Pancreas and colon tissue were analyzed by flow cytometry, immunohistochemistry and electron microscopy from WT, CST-KO and CgA-KO mice. The direct effects of CgA and CST on leukocyte recruitment were determined *in vivo* in mice and *in vitro* on mouse and human cells.

Results CST seems to plays a prominent role in intestinal health since we observed that CST-KO mice are displayed with colon inflammation and a “leaky’ gut. To determine the role of CST in the leukocyte infiltration, we studied the chemotactic abilities of CST using leukocyte recruitment assays. Finally, blood plasma CgA levels suggest that the contrasting activities of CgA and CST link to IBD activity.

Conclusions Here we show that CgA and its counteracting cleavage product CST are key modulators of intestinal health and disease. The observed inflammatory complications in IBD patients seem to depend on the balance between local and circulating CgA and CST levels, where CgA contributes to an inflammatory environment and CST seems to dampen or contributes to resolving the inflammation. Our study shows that CgA and CST levels are not only relevant markers for disease severity of IBD, but suggests that they might also be targets for therapeutic interventions in inflammatory diseases.

Dissecting the functional properties of serotonin release from "the other" chromaffin cells in the gut

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Enterochromaffin (EC) cells form a group of intestinal stem-cell derived secretory cells in the gut epithelium that respond to nutrients, bacterial metabolites, and mechanical stimulation. EC cells are found in all regions of the gut, express genes encoding for proteins of the presynaptic release machinery (Bellono et al., *Cell*, 2017), and co-release different peptide hormones and serotonin (5-HT). In the duodenum, 5-HT serves as a fast-acting satiety signal that is transmitted to the brain via the vagus nerve, while in the colon, 5-HT regulates gut motility. Despite the important role of gut-derived 5-HT in physiology and gut-brain-signaling, the functional properties and molecular mechanisms that determine vesicle fusion in EC cells are virtually unknown. To address these questions, we recently established a multidisciplinary experimental approach combining mouse genetics, 2D monolayer and 3D organoid cultures, electron microscopy, and single-cell electrochemistry to study mouse EC cells *in vitro* (Shaaban et al., BioRxiv, 2021; https://doi.org/10.1101/2021.05.28.446100). Using single-cell carbon fiber amperometry, we found that EC cells release the majority of 5-HT from large dense core vesicles with kinetics comparable to those of catecholamines from adrenal chromaffin cells. To dissect functional vesicle pools in EC cells, we measured changes in membrane-capacitance in response to a series of depolarization steps and compared cultured duodenal and colonic EC cells. Our data indicate, that while the amount of 5-HT released from individually fusing vesicles is the same for cells from both gut regions, the total amount of vesicles that fuse in response to a series of depolarization pulses differs dramatically. In summary, we present a methodological workflow that provides very standardized experimental conditions for the analysis of 5-HT release from individual EC cells, and that can be used to dissect regional differences in functional properties between cells and - ultimately - the molecular determinants governing vesicles exocytosis.

Nano-biomolecular condensates control the clustering of synaptic vesicles

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Recycling synaptic vesicles are responsible for neurotransmission, and they cluster with the reserve pool of vesicles at the presynapse (Rizzoli and Betz, *Science* 2004). More recently, the presynaptic protein synapsin was found to form biomolecular condensates *in vitro* and hypothesized to control synaptic vesicle clustering (Milovanovic et al., *Science* 2018). However, no direct visualisation of these condensates in live synapses has been possible so far, because of the small and highly crowded nature of the presynaptic microenvironment.

We took an unbiased mass spectrometry approach to identify presynaptic candidates for their ability to sense synaptic activity (via phosphorylation) and to undergo liquid-liquid phase separation (via disordered domains). We identified several proteins, including synapsin and, surprisingly, the microtubule associated protein Tau, a major player in dementia pathology. As Tau was recently shown to bind synaptic vesicles (Zhou et al., *Nature Communications*, 2017; McInnes et al., *Neuron*, 2018), we investigated its potential presynaptic role. Traditionally, fluorescent recovery after photobleaching is used to identify biomolecular condensates in cells, including Tau (Wegmann et al., *EMBO J.,* 2018). However, the small size of the synapse precludes using this indirect method to assess the reduced mobility inherent to molecules undergoing liquid-liquid phase separation. We took advantage of single molecule super-resolution microscopy to directly track Tau molecules in conjunction with various pools of synaptic vesicles in live hippocampal neurons (Joensuu et al., *Nature Protocols*, 2017). Using live hippocampal neurons from either MAP-Tau knockout mice neurons in rescue conditions or TALEN gene-edited Tau-mEos2 mice, we discovered that Tau is necessary and sufficient to selectively control the nanoscale mobility of the recycling pool of synaptic vesicles. We used two-colour single molecule tracking and various pharmacological treatments to reveal that this effect relies on the formation of nanoscale biomolecular condensates at the presynapse that intermix with the vesicle pools in an activity-dependent manner.

Imaging the nanoscale structure of dense core vesicles with correlative super-resolution light and 3D electron microscopy.

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The plasma membrane separates the cell's interior from the outside world. The exchange of signals and material across this barrier is regulated by a multitude of channels, transporters, receptors, and trafficking organelles. Mapping the molecular structure and dynamics of the plasma membrane is key to understanding how human cells function in health and malfunction in disease. Electron microscopy can produce high resolution images of the membrane. Yet, it has been challenging to locate and identify proteins within these images. Recently-developed super-resolution localization microscopy, however, can image fluorescently-labeled molecules with better than 20 nm precision but lacks cellular context provided by EM. We developed a correlative super-resolution light and EM method (CLEM) that couples these complementary methods to produce images where identified proteins are mapped within the dense native environment of the plasma membrane at the molecular scale. This correlative method is uniquely suited to determine the nanometer-scale organization of the plasma membrane and associated organelles. To improve our resolutions and map proteins in 3D directly around organelles, we next developed a complementary and targetable genetically-encoded electron microscopy labeling method that uses histidine-based affinity tags and metal-binding gold-nanoparticles to mark the location of proteins using electron tomography. The combination of these two methods allows us to locate specific proteins directly across the plasma membrane at single organelles of excitable cells.

In endocrine cells and neurons, dense core vesicles are transported to the plasma membrane where they dock and prepare for exocytosis. Rab-GTPases and their interacting partners are key regulators of this process. Where and how these proteins are positioned and organized with respect to the vesicle and plasma membrane are mostly unknown. First, we use correlative super-resolution light and platinum replica electron microscopy to map Rab-GTPases (Rab27a and Rab3a) and their effectors (Granuphilin-a, Rabphilin3a, and Rim2) at the nanoscale in 2D. Next, we employ our genetically-encoded electron microscopy labeling method to determine the axial location of exocytic proteins using electron tomography. These data show that Rab-GTPases and their effectors are distributed across the entire surface of individual docked vesicles. This circumferential distribution likely aids in the efficient transport, capture, docking, and rapid fusion of vesicles in excitable cells.

Currently, gaps exists between understanding protein structures and their cellular contexts. Our studies aim to fill these gaps by developing and using new ultra-high resolution imaging tools to determine the nanoscale organizations and dynamics of molecules that are important for membrane traffic. As we piece together structures in their specific cellular contexts, a new holistic understanding of how biological processes work will be gained. These studies will help to map the fundamental architecture of cellular machines to better understand how these complex assemblies function in healthy cells and malfunction in disease.

Optimized Nanoscale Expansion Microscopy

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Super-resolution imaging applications overcoming Abbe's criteria have been applied to biological specimens for more than two decades. Nevertheless, such technologies have major difficulties in reaching the single-digit nanometer scale, with only a few highly complex technologies providing imaging precision beyond ~5 nm. Here we provide an easy, inexpensive, and effective solution to this problem, in the form of optimized nanoscale expansion (*ONE*) microscopy, in which we combine the 10-fold physical expansion of the specimen with optical super-resolution technology. This approach reaches easily the 5 nm domain, and is readily applied to imaging molecular complexes or individual molecules in their native cellular/tissue environment, both in cell culture and in tissue. *ONE* microscopy described molecular complexes, solved long-standing questions and revealed novel structures, implying that this is a promising approach for future research and disease diagnostic applications. Its introduction should be especially facile, since *ONE* microscopy does not require prior extensive imaging knowledge and it is applicable to multiple existing microscopy setups and expansion protocols.

Molecular resolution fluorescence imaging

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In the last decade, super-resolution microscopy has evolved as a very powerful method for sub-diffraction resolution fluorescence imaging of cells and structural investigations of cellular organelles. Super-resolution microscopy methods can now provide a spatial resolution that is well below the diffraction limit of light microscopy, enabling invaluable insights into the spatial organization of proteins in biological samples. However, current super-resolution measurements become error-prone below 25 nm. In addition, refined single-molecule localization microscopy methods achieved localization precisions of only a few nanometers, but here too translation of such high localization precisions into sub-10 nm spatial resolution in biological samples remains challenging. In my contribution I will discuss two possibilities to bypass these limitations. One is based on physical expansion of the cellular structure by linking a protein of interest into a dense, cross-linked network of a swellable polyelectrolyte hydrogel. Since its first introduction by Boyden and co-workers in 2015, expansion microscopy (ExM) has shown impressive results including the magnified visualization of pre- or post-expansion labeled proteins and RNAs with fluorescent proteins, antibodies, and oligonucleotides, respectively, in cells, tissues, and human clinical specimen. By combining ExM with super-resolution microscopy it is potentially possible to enable multicolor molecular resolution fluorescence imaging. The other approach uses resonance energy transfer between fluorophores separated by less than 10 nm and reveals interfluorophore distance information from time-resolved fluorescence detection in combination with photoswitching fingerprint analysis. We will show how the method can be used advantageously to determine the number and distance even of spatially unresolvable fluorophores in the sub-10 nm range.

VAMP2 AND SYNAPTOTAGMIN MOBILITY IN CHROMAFFIN GRANULE MEMBRANES: IMPLICATIONS FOR REGULATED EXOCYTOSIS

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Granule-plasma membrane docking and fusion can only occur when proteins that enable these reactions are present at the granule-plasma membrane contact. Thus, the mobility of granule membrane proteins may influence docking, and membrane fusion. We measured the mobility of vesicle associated membrane protein 2 (VAMP2), synaptotagmin 1 (Syt1), and synaptotagmin 7 (Syt7) in chromaffin granule membranes in living chromaffin cells. We used a method that is not limited by standard optical resolution. A bright flash of strongly decaying evanescent field produced by total internal reflection (TIR) was used to photobleach GFP-labeled proteins in the granule membrane. Fluorescence recovery occurs as unbleached protein in the granule membrane distal from the glass interface diffuses into the more bleached proximal regions, enabling the measurement of diffusion coefficients. We found that VAMP2-EGFP and Syt7-EGFP are mobile with a diffusion coefficient of approximately 3 x 10^{-10} cm^2/s. Syt1-EGFP mobility was below the detection limit. Utilizing these diffusion parameters, we estimated the time required for these proteins to arrive at docking and nascent fusion sites to be many tens of milliseconds. Our analyses raise the possibility that the diffusion characteristics of VAMP2 and Syt proteins could be a factor that influences the rate of exocytosis.

α-Synuclein kinetically regulates the nascent fusion pore dynamics

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α-Synuclein has for a long time been known infamously for its role in progression of Parkinson's disorder. Even though it forms 1% of the total proteome in neurons, its physiological function at the presynapse is largely allusive. Recent studies suggest conflicting role of α-Synuclein in modulating release with few studies suggesting it has inhibitory effect while some suggesting stimulatory role. In our study we used BLM electrophysiology to study the effect of α-Synuclein on the sub-millisecond dynamics of single molecule fusion pore. Transient fusion pores formed during exocytosis is a crucial intermediate that controls cargo release from vesicles. We show that α-Synuclein has an inhibitory effect on secretion by decreasing the open probability of fusion pores. This inhibitory effect is dependent on the ability of α-Synuclein to bind to membranes containing negatively charged phospholipids. α-Synuclein has been previously shown to interact with the v-SNARE (Syb2). It accessed Syb2 in SNARE complexes stoichiometrically to close pores as we found that increasing Syb2 copy number required increasing α-Synuclein concentrations to fully close pores. Interestingly, α-Synuclein's inhibitory function was seen even in the presence of SNARE chaperones Munc13 and Munc18 and it promoted membrane fusion clamping activity of calcium sensor Synaptotagmin-1 (apo condition). In the presence of calcium - Synaptotagmin-1 was able to keep the pore stably open even in the presence of high concentrations of α-Synuclein, thus preventing the inhibitory activity of α-Synuclein. Thus, this study provides an explanation for the conflicting results obtained in previous studies on α-Synuclein wherein we propose that α-Synuclein has an inhibitory effect on vesicular secretion which is lost during calcium influx.

Munc13 regulates exocytosis by two distinct Ca^{2+}-dependent mechanism.

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Munc13 is a Ca^{2+} and DAG-dependent cytosolic protein that is essential for priming of secretory granules and synaptic vesicles for exocytosis. The protein contains an elongated SNARE-interacting MUN domain that is flanked by DAG/Ca^{2+}-binding C1 and C2 domains. To understand how the C2B domain contributes to recruitment of Munc13 to the release site and to priming, we quantified (EGFP-labelled) Munc13 translocation and exocytosis in insulin secreting INS1 cells, by combined TIRF microscopy and patch clamp electrophysiology. In response to increased intracellular Ca^{2+} or DAG, Munc13 translocated to the plasma membrane (within 1 and 22 s, respectively) and accumulated at docked granules. Granules undergoing stimulated exocytosis harbored twice as many Munc13 copies than those that failed to do so (16 vs 8 molecules), suggesting that accumulation occurred during priming. In addition, we found transient Ca^{2+}- and Munc13-dependent facilitation of exocytosis 500-1000 ms after Ca^{2+}-influx, which was unrelated to translocation of the protein. A Munc13-C2B domain mutant that mimics the Ca^{2+}-bound form (Munc13-DDNN) was constitutively bound to the membrane, and led to priming that was independent Ca^{2+} and DAG. Another mutant in loop 3, predicted to disrupt Ca^{2+}-dependent helix insertion (Munc13-loop3AAA), translocated in response to increased DAG; facilitation, but not priming was lost by expression of Munc13-loop3AAA. We conclude that Ca^{2+}-binding to the C2B domain promotes exocytosis by two distinct mechanism, 1) slow translocation of Munc13 to the release site, and 2) a fast-reversible switch that acts at the assembled exocytosis machinery. The low copy number of Munc13 at the release site suggests that the protein limits priming by engaging stoichiometrically with preformed SNARE complexes.

Munc18 isoforms differentially support docking and priming of insulin granules

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The SM-protein Munc18 and its binding partner syntaxin are crucial for two distinct steps of regulated exocytosis, docking of secretory granules at the plasma membrane and priming reactions that prepare docked granules for Ca^{2+} dependent membrane fusion. Both proteins co-cluster at the docking site when a granule arrives at the plasma membrane. To study the mechanism and isoform-dependence of these separate munc18 functions, we generated knockouts of munc18-1 (1KO), munc18-2 (2KO), or both (DKO) in insulin-secreting INS1 cells, and quantified granule docking, priming, and protein clustering by TIRF imaging and capacitance recordings. Surprisingly, docking defects were subtle; even in the DKO, the number of near-membrane granules was unaffected, but motion of granules was increased and undocking occurred more frequently than in control. In the 1KO, priming and exocytosis were strongly decreased, with little effect on docking. In contrast, in the 2KO priming was intact, while the effect on docking was stronger than in 1KO. Expression of either munc18-1or munc18-2 rescued docking and exocytosis, as did expression of mutants that weaken binding to syntaxin (EA and EK mutants). The four proteins accumulated at docked granules, with affinities decreasing in the order munc18-1>EA>EK>munc18-2. Clustering of syntaxin-1, but not syntaxin-3, at granules was strongly impaired in all three knockout lines. Finally, single molecule imaging demonstrated that syntaxin-1 and Munc18-1 exhibit similar diffusion behavior, and pairs of the two proteins were observed to travel together in the membrane. We conclude that both munc18 isoforms contribute to granule docking, likely by promoting syntaxin-1 clustering at the docking site, while priming relies on munc18-1. Thus, the data suggest the separate functions of munc18 during docking and priming are supported by different isoforms of the protein.

Noradrenergic suppression of IFNγ-induced lysosomal deposition of MHC class II molecules at the surface of rat astrocytes

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Astrocytes are abundant neuroglial cells in the central nervous system (CNS) that maintain homeostasis and defend the CNS from insults. Initiation of reactive astrogliosis, a universal response to pathological conditions, contributes to neuroinflammation. In astrocytes, pro-inflammatory cytokine interferon γ (IFNγ) induces surface expression of the major histocompatibility complex class II (MHCII) molecules involved in antigen presentation. This multistage process includes lysosomal exocytosis of MHCII at the plasma membrane. Noradrenaline (NA) is a potent anti-inflammatory mediator and here we investigated the mechanism of MHCII deposition at cell surface in IFNγ-treated astrocytes by adrenergic mechanisms by monitoring vesicle fusion by membrane capacitance, super-resolution and confocal microscopies with immunocytochemistry using antibodies against MHCII. Moreover, we stimulated reactive astrocytes in culture with non-specific (noradrenaline) or specific (isoprenaline, phenylephrine) adrenergic agonists, in conjunction with specific antagonists (terazosin, propranolol), or with a cell-permeable synthetic analog of cAMP (dbcAMP). The results revealed that IFNγ-treated astrocytes increased the expression and fusion of lysosomes. Selective agonist of β-, but not of α-adrenergic pathway strongly suppressed surface expression of MHCII in reactive astrocytes. Similarly dbcAMP co-applied to IFNγ-treated astrocytes suppressed MHCII surface expression. Our results indicate that noradrenaline-mediated suppression of MHCII surface expression is principally mediated via β-adrenergic pathway involving lysosomal compartments.

$Ca_V1.3$ L-type Ca^{2+} channel modulates pancreatic β-cell electrical activity and survival.

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Pancreatic β-cells express several high voltage-gated Ca^{2+} channel isoforms critical for insulin release, cell differentiation, and survival. RNAseq and qPCR analyses demonstrated that $Ca_V1.3$ L-type Ca^{2+} channel is highly expressed in pancreatic islets of both mice and men. Moreover, genetic polymorphisms leading to loss-of-function associate with increased susceptibility for diabetes while $Ca_V1.3$ gain-of-function mutations cause hyperinsulinaemic hypoglycemia in humans. Nevertheless, functional evidence for the physiological role of $Ca_V1.3$ is contradictory and largely unknown. Here we show that $Ca_V1.3$ deletion led to a 6-fold increase in DNA damage and a 3-fold decreased proliferation markers in pancreatic β-cells of 14-days old mice, while adult mice were largely unaffected. However, β-cell mass was reduced by ~20% in both young and old mice. Functionally, $Ca_V1.3$ deletion led to similar effects in both ages. Voltage-clamp recordings in β-cells of 14-days old mice showed a ~20% reduction in whole-cell Ca^{2+} influx (WT I_{peak} = -19.8±1.0 pA/pF, $Ca_V1.3^{-/-}$ I_{peak} = -14.8±0.6 pA/pF) accompanied by slower activation and inactivation kinetics as well as a ~5mV rightwards shift of the voltage-dependence of activation (WT $V_{1/2}$ = -7.7±0.8 mV, $Ca_V1.3^{-/-}$ $V_{1/2}$ = -2.3±1.1 mV). Moreover, current-clamp recordings showed that $Ca_V1.3$ deletion delayed the glucose-induced action potential (AP) onset, reduced AP firing frequency (in 7.5mM glucose WT= 4.3Hz, $Ca_V1.3^{-/-}$= 2.1Hz) and AP-train frequency (in 7.5mM glucose inter-train interval WT= 49.3±9.6 sec, $Ca_V1.3^{-/-}$= 120.3±25.5 sec). AP-clamp experiments indicated that these effects were caused by reduced pace-making current at the beginning of an AP-train as well as during repetitive stimulation due to reduced voltage-dependent facilitation. Therefore, our data demonstrate that the $Ca_V1.3$ channel is important for postnatal β-cell survival and proliferation, contributes to Ca^{2+} influx, is required for the initiation of glucose-induced electrical activity and thereby modulates insulin release both at low and high glucose concentrations. Support: FWF P31434, DOC30-B30.

Molecular mechanisms responsible for the sexual dimorphism in pancreatic β-cell insulin release

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In humans, Type 2 Diabetes Mellitus (T2DM) has a higher incidence in males compared to females, a phenotype recapitulated by many rodent models. While the sex difference in insulin sensitivity partially accounts for this phenomenon, hitherto uncharacterized differences in pancreatic β-cell insulin release strongly contribute. Here we show that stepwise increase in extracellular glucose concentration (2, 5, 7.5, 10, 15, 20 mM) induced electrical activity in β-cells of both sexes with similar glucose sensitivity (Female EC_{50} =9.45±0.15 mM, Male EC_{50} =9.42±0.16 mM). However, female β-cells resting membrane potential (RMP) and inter-spike potential (IP) were significantly higher compared to males (e.g.@15mM glucose: Male RMP= -82.7±6.3, IP= -74.3±6.8 mV, Female RMP= -50.0±7.1, IP= -41.2±7.3 mV). Females also showed higher frequency of trains of action potential (AP) (@10mM glucose: Male F= 1.13±0.15 trains/min, Female F= 1,78±0.25 trains/min) and longer AP-burst duration (e.g.@10mM glucose: Male 241±30.8ms, Female 419±60.2ms). The higher RMP in females reduced the voltage-gated calcium channel (Ca_V) availability by ~60%. This explains the paradoxical observation that despite identical Ca_V expression levels and higher electrical activity, the islet Ca^{2+} transients were smaller in females compared to males. Interestingly, the different RMP is not caused by altered K_{ATP}, TASK, or TALK K^+ currents. However, Stromatoxin-1-sensitive $K_v2.1$ K^+ current amplitude was almost double in males (I_K = 130.93±7.05pA/pF) compared to females (I_K = 75.85±11.3pA/pF) when measured at +80mV. Our results are in agreement with previous findings showing that $K_v2.1$ genetic deletion or pharmacological block leads to higher insulin release and β-cell survival. Therefore, we propose the sex-specific expression of $K_v2.1$ to be the mechanism underlying the observed sexual dimorphism in insulin release and the incidence of T2DM. Support: FWF P31434 and DOC30-B30.

Investigating the Role of Synaptobrevin2 dimers in functional SNARE complex assembly

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A synchronized overall function of human body requires precise spatiotemporal regulation of the release of chemical messengers by cells, via the process called exocytosis. These chemical messengers (e.g. hormones, neurotransmitters, peptides) are present within vesicles, which fuse with the plasma membrane to release their cargo into the extracellular space. SNARE (Soluble N-ethylmaleimide–sensitive factor Attachment protein Receptor) proteins, present on the vesicle (v-SNARE) and on the target membrane (t-SNARE), catalyze membrane fusion; which is precisely controlled by regulatory proteins. These proteins bridge the gap between the two opposing membranes and initiate membrane fusion, leading to the formation of ephemeral fusion pore - the first aqueous connection between vesicular lumen and extracellular fluid. SNARE along with lipid molecules constitute the minimal machinery essential for membrane fusion. V-SNARE protein known as Synaptobrevin2 (Syb2) is one of the most abundant proteins on synaptic vesicles (~70 copies of Syb2/ synaptic vesicle). On the other hand, many studies show a variable syb2 copy number (3-15) required for membrane fusion. Previous studies have also shown that Syb2 dimerize through its transmembrane domain and these dimers are hypothesized to have a structural role in fusion pore. But the exact physiological role of Syb2 dimerization in membrane fusion is unknown. Here, we are investigating the role of Syb2 dimers in functional SNARE complex assembly. We have used an in-vitro reconstitution-based approach and synaptic vesicles isolated from rat brain to characterize Syb2 dimers using different ensemble and single molecule fusion assays. We found that Syb2 density, Syb2 copy number, lipid environment attribute to Syb2 dimerization. Overall our results suggest the possibility of dimer being a structural part of functional SNARE complex assembly.

Genetic deletion of Stac2 adaptor protein alters electrical activity of mouse chromaffin cells

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Voltage-gated L-type Cav1.2 and Cav1.3 calcium channels (LTCC) regulate chromaffin cell action potential (AP) firing and catecholamine secretion. Src homology 3 and cysteine rich domain adaptor proteins (Stac) have been recently identified as novel regulators of LTCC membrane trafficking and gating. Upon overexpression in cultured hippocampal neurons or heterologous expression systems the Stac2 isoform abolishes LTCC calcium dependent inactivation via an allosteric inhibition of calmodulin binding. Additionally, human STAC2 mutations have been identified in patients with schizophrenia and in Drosophila deletion of the homologous DStac gene results in deficient LTCC calcium transients and reduced neuropeptide release. Our quantitative RT-PCR analyses shows that Stac2 mRNA is expressed in mouse chromaffin cells (MCCs). Thus, the aim of this study was to investigate the effect of Stac2 deletion on calcium currents and MCC electrical activity.

Constitutive genetic ablation of Stac2 does not affect the resting membrane potential and spontaneous firing frequency of MCCs. However, the AP depolarization threshold was significantly reduced in Stac2$^{-/-}$ MCCs compared to WT. Additionally, step current injection showed that Stac2$^{-/-}$ MCCs responded with an increased AP firing frequency at the onset of the pulse (F_0) but went faster into depolarization block. Surprisingly, initial characterization of LTCC biophysical properties suggests that the enhanced electrical activity observed in Stac2$^{-/-}$ MCCs cannot be explained by altered calcium currents. We currently investigate possible effects of Stac2 deletion on other ionic conductances governing MCCs excitability, and catecholamine vesicle exocytosis.

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Newly synthesized chromaffin secretory vesicles are preferentially released.

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Chromaffin cells are the parenchymal secretory cells of the adrenal medulla that synthesize, store and secrete a cocktail of small molecules, such as catecholamines, ATP, and also bioactive peptides as NPY, -granins, and enkephalins.

Chromaffin cells transfected with neuropeptide Y (NPY-EGFP) release EGFP almost exclusively in the first secretory stimuli. This indicates that newly-synthesized secretory vesicles (SVs) are preferentially secreted over the older ones (Estévez-Herrera et al., 2016). This also suggests that distinct populations of SVs containing new and old cargoes (catecholamines and peptides) that are differentially competent to carry out exocytosis.

In this study, we have tried to address the question of whether new vesicles have a high probability to be released first. Also, we have asked which role have the vesicles that are not competent to exocytosis.

Our results show the preferential release of newly synthesized NPY-Halo in the PC12 cells, using a combination of multi-labeling systems and compared its release with an endogenous releasable protein chromogranin B.

Also, we have tested if this new SVs preferentially uptake and store more dopamine (and serotonin) than old vesicles. Online secretion and single-cell amperometry show an increase of dopamine secretion in the first pulses that correspond with an increase in the quantal size.

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Analysis of a HSPB1 mutation in Charcot-Marie-Tooth disease using patch-clamp electrophysiology of bovine chromaffin cells

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Charcot-Marie-Tooth (CMT) disease is one of the most commonly inherited progressive neurological disorders, characterised by muscle degeneration and neurosensory deficits caused by mutations in neuronal proteins. The majority of cases (50-70%) of CMT disease are categorised into type 1 (demyelinating) and type 2 (intra-axonal dysfunction). Patients diagnosed with CMT type 2F (CMT2F) caused by dominant mutations in the small heat shock protein beta-1 (HSPB1) display progressive weakness within the distal muscles of the arms and legs. Genetic mutations within the HSPB1 gene have been identified as specific causative factors in CMT type 2; however, the mechanisms by which these mutations create progressive neuronal defects are largely unknown. HSPB1 is an ATP-independent chaperone that regulates proteostasis through dynamic interactions with client proteins. Structurally, HSPB1 consists of an alpha-crystallin domain flanked by N and C termini. Mutations in the alpha-crystallin domain have been the subject of many studies. In this project we focused on the C-terminal HSPB1 Q175X mutation first identified in a family of CMT-affected patients. Using the *C. elegans* nematode model, we expressed either wild-type (WT) human HSPB1 or HSPB1 Q175X specifically in neurons. In the behavioural aldicarb assay (a proxy assay for analysing synaptic transmission) we found that expression of the Q175X mutation caused a resistance to the acetylcholinesterase inhibitor aldicarb that changed progressively with *C. elegans* age. To examine the mechanisms underlying progressive neurotransmission dysfunction in response to the Q175X mutation further, we are comparing the effects of overexpression of wild type and mutant protein on stimulus-evoked exocytosis in bovine chromaffin cells using whole-cell patch-clamp recording and membrane capacitance measurements. Specifically, we are investigating potential defects in vesicle docking and exocytosis.

GLUCOSE UPTAKE IN THE ENDOCRINE PANCREATIC CELLS THROUGH GLUCOSE TRANSPORTERS

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About 422 million people in the world are affected by diabetes. The most common forms of diabetes are non-insulin-dependent diabetes (T2D). Glucose homeostasis is regulated via glucose transporters (GLUTs). In the human pancreatic cells, GLUT1 and GLUT2 have a great role in the development of diabetes. The aim of this study: 1) To identify candidate proteins involved in GLUTs internalization. 2)To determine the effect of different glucose concentrations on GLUT transporter. 3) To verify the expression of these proteins involved in GLUT transporter in human pancreatic cells. 4) To understand how the expression of GLUT transporters involved in glucose transport is altered in type-2-diabetic cells. INS1 cells were tagged with GLUT1 and GLUT2 GFP, Rab5, endophilin, and clathrin to study trafficking events. TIRF and confocal microscopy techniques were used for studying the trafficking of GLUTs. qPCR was performed to understand the relative expression of GLUTs in diabetic and non-diabetic conditions. Tagged transporter was found to co-localize with proteins like Rab5, endophilin, and clathrin during the transporter trafficking process. The trafficking increased with glucose concentrations showing a higher number of GLUT1 and GLUT2 vesicles. This increase was coupled with increased co-localization of proteins involved in trafficking such as clathrin. Expression of GLUT1 transporters was decreased in T2D human islets but GLUT2 expression remained unaffected. Clathrin co-localized with GLUT2 in human T2D cells due to no decrease in their expression under such conditions. This shows the importance of GLUT transporter trafficking in maintaining glucose homeostasis and how it is affected in T2D.

An integrated system for quantifying chromaffin cell secretion

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We have created a four-channel compact computer-operated system for the analyses of secretory products from perifused bovine chromaffin cells or perfused rat adrenals.

We have also designed a novel multichannel digital stimulator for delivering electrical pulses to a low impedance like perfused adrenal glands (CanStim-8). The system integrates chemical stimulation or electrical stimulation, on-line amperometrical recording of secreted catecholamines and a fraction collector for the analysis of other secreted substances (ATP, ascorbate, chromogranins, peptides).

Therefore, we have created: i) an 8-channels digital stimulator, ii) an 8-channels electronic valve controller, iii) cell bed perifusion chambers, iv) electrochemical cells, v) a four-channel potentiostat and vi) a parallel fraction collector.

The main goal is having a whole system that can perform whole experiments on adrenal secretion automatically, in response to short (2-5 s) secretagogue pulses or electrical stimuli. The system simultaneously records the four-channel responses using novel electrochemical cells coupled to a four-channel potentiostat (CANSTAT-4). The effluent is then directly collected in a tailoring-made fraction collector (CANSCollect).

The whole system is controlled through a LabChart for PowerLab (ADInstruments, Dunedin, NZ), which take the analogic signals from the catecholamine detection and, through TTL signals, also triggers the electronic valves and the fraction collector.

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Effect of reversible SERCA blockade on depolarization-induced CA exocytosis in chromaffin cells from normotensive Wystar Kyoto rats and Spontaneously Hypertensive Rats

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Spontaneously hypertensive rats (SHR) and their genetic counterpart, the normotensive Wistar Kyoto rats (WKY) are widely used in the study of the genesis of essential hypertension. Adrenomedullary chromaffin cells (CCs) have been involved in this pathology since in SHR these cells secrete about two times more catecholamine (CA) after 12 weeks of age compared to WKY rats

In a recent study, Martínez-Ramírez et. al. [doi.org/10.1007/s00424-020-02483-1] reported that the endoplasmic reticulum (ER) of mouse CCs functions globally more like a "Ca^{2+} sink" in and bovine CCs more like a "Ca^{2+} source". Consequently, mouse CCs display weaker Ca^{2+}-induced Ca^{2+}-release (CICR) and strong Ca^{2+} sinking whereas the reverse is true for bovine CCs. Our purpose here is to determine which of these "modalities" applies to rat CCs and how SHR CCs compare to WKY CCs, given that they display quite a different gain of CICR. Intracellular Ca^{2+} handling by the ER was examined indirectly by measuring, using single-cell amperometry, CA secretion elicited by a series of depolarizing pulses before and after the reversible blockade of the ER Ca^{2+} ATPase (SERCA) with cyclopiazonic acid (CPA).

CCs from 12 to14 week-old WKY and SHR rats were challenged with high K^+ pulses separated by 30 sec intervals. CPA (5 uM) was superfused during the 3^{th} to 5^{th} pulse, while a carbon-fiber microelectrode, placed near the CC membrane recorded amperometric spikes to quantify CA secretion. The number of spikes and the cumulative charge reached after each pulse were compared between pulses and in CCs from either WKY or SHR.

We found that in CCs from both WKY and SHR, the ER behaves more like a "Ca^{2+}-source", although SHR CCs secrete significantly more CA than WKY CCs. This suggests that ER Ca^{2+} handling of CCs from both rat strains resembles more that of the evolutionary more distant bovine than the mouse.

Neuronal SNARE assembly enlightened by cryo-EM structures of a synaptobrevin-Munc18-1-syntaxin-1 complex

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Neurotransmitters are released by tightly controlled synaptic vesicle exocytosis at the active zone of presynaptic nerve terminals. This process requires primed fusion-competent vesicles and coordinated calcium-secretion coupling, and all these steps are governed by a sophisticated protein apparatus. SNAREs form SNARE complexes that bridge the vesicle and plasma membranes, triggering neurotransmitter release. Productive formation of SNARE complexes is topologically complicated and requires overcoming multiple energy barriers. Munc18-1 forms a template to organize assembly of the SNARE complex, binding first to a closed conformation of syntaxin-1 where its N-terminal region interacts with the SNARE motif, and later binding to synaptobrevin. However, the mechanism of SNARE complex assembly remains enigmatic. Now we report two cryo-EM structures of Munc18-1 bound to cross-linked syntaxin-1 and synaptobrevin. The structures allow visualization of how syntaxin-1 opens and reveal how part of the syntaxin-1 N-terminal region can help nucleate interactions between the N-termini of the syntaxin-1 and synaptobrevin SNARE motifs while their C-termini bind to distal sites of Munc18-1. These observations, together with mutagenesis, SNARE complex assembly experiments and fusion assays, support a model whereby these interactions are critical to initiate SNARE complex assembly and multiple energy barriers enable diverse mechanisms for exquisite regulation of neurotransmitter release.

Impairment of cellular maturation dynamics in early stages of ALS lead to alterations of exocytosis in the SOD1^{G93A} mouse

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In amyotrophic lateral sclerosis (ALS) there is a synaptic dysfunction in the motor neuron (MN), which is characterized by affecting some cytosolic fundamental elements for a healthy exocytosis, for example the $[Ca^{2+}]_c$ or the cellular organelles that regulate its concentration. In recent years, it has been demonstrated that cells affected in ALS are not limited to MN. Cells as diverse as liver cells or the chromaffin cells (CC) of the adrenal medulla have also been implicated. CCs are a good model to study exocytosis and synaptic dysfunction in ALS. Therefore, the objective of this study is to determine in SOD1^{G93A} mouse, if synaptic alterations can be observed in CCs in the early stages of life (30 days postpartum; P30) that might help to understand the onset of ALS. In the present study we have observed a subtle change in the exocytosis kinetics when the cell is stimulated with 75 mM K^+ ($75K^+$), being affected in relation to WT: the I_{max} (36.5 *vs* 30.2 pA; $p < 0.01$), rise rate (14.4 *vs* 10.9 pA/ms; $p < 0.01$), and fall time (5.9 *vs* 7.0 ms; $p < 0.05$). However, the amperometric charge (pC) of the spike and the number of exocytosis events are not affected. If we analyze those key elements that participate in exocytosis, there is a change in the kinetic regulation of $[Ca^{2+}]_c$. These changes are attributed in part to mitochondrial damage (ultrastructural aberrations). Consequently, Ψ_m and its maximum respiration are also affected, as well as the global ATP content of the adrenal gland. This matches with what was observed in microscopy studies, where the SOD1^{G93A} mouse shows with respect to the WT: **i)** a gradual reduction in the distribution of secretory vesicles, being ~25 % lower the number of total vesicles, but ~52% in the environment of 1.5 µm close to membrane; **ii)** the vesicles that have a typical adrenergic morphology are smaller, without their cell density being affected; **iii)** on the contrary, the characteristic rod-like vesicles have the same size, but have lower density (~ 1 vs 1.5 vesicles/µm^2); **iv)** higher vesicle cargo, but fewer immature vesicles in the vicinity of the dilated ER; **v)** the presence of the SOD1^{G93A} protein was detected inside the vesicles; **vi)** the measurement of overall gland content of neurotransmitters by HPLC did not detect differences in their concentrations. Finally, **vii)** cytoskeleton analysis showed an increase in actin cell volume (347.5 *vs* 248.0 µm^3); however, this contrasts with its inability to polymerize into F-actin.

In conclusion, the CC of SOD1^{G93A} ALS mouse at early pre-disease ages (P30) shows subtle kinetic changes in relation to exocytosis and its ability to regulate $[Ca^{2+}]_c$. However, it has serious ultrastructural changes related to the maturity and dynamics of its cellular organelles that are likely caused by damage to the cytoskeleton.

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COMPARISON OF DIFFERENT FORCE FIELDS IN MD SIMULATIONS OF ION-MEMBRANE INTERACTIONS

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Molecular dynamics (MD) simulations of lipid membranes have been widely used to better understand phenomena at the atomic and molecular level that cannot be observed with conventional experimental methods. This technique is very useful to understand the underlying mechanisms of the most common biological ions, such as K^+, Na^+, Cl^- or Ca^{2+} when they interact with the lipid bilayer or when they go through the induced pores during electroporation. Lipid bilayer simulations showed that K^+ and Cl^- interact very little with the lipids, compared to Na^+ and Ca^{2+}. With the tools of MD, ion-lipid bilayer interactions can be studied and analyzed under a wide range of conditions: number and types of lipids, number and types of ions, physical conditions, thermodynamic ensembles, barostats, thermostats, etc. We studied lipid bilayer systems in the presence of different salts, such as the monovalent KCl and NaCl, and the divalent $CaCl_2$, using different force fields. As pointed out by other groups, the Ca^{2+} and Na^+ models, built-in and distributed with the Standard CHARMM36 force field, do not describe the real interaction between ions-ions and ions-water molecules, overestimating the interaction between ions and water and underestimating the interaction between cations and Cl^-. The serious discrepancies between the behavior of the standard force field ion models in aqueous solution and the experimentally determined properties of the hydrated ions, lead us to compare different force fields (GROMOS-OPLS and CHARMM36) and ion models (standard, electronic continuum correction - ECCR, and Non-Bonded FIX - NBFIX) in a lipid-aqueous environment, to obtain more realistic interactions between ions, water, and phospholipids. We evaluated the artificial ion-ion clustering, the ion-lipid binding, the resident time, and other several parameters in pure POPC or POPC-POPS systems, in order to compare the different conditions simulated.
Overall, among all the Ca^{2+} ion models, the ECCR correction applied to CHARMM36 force field is the model that best represents ion-ion, ion-water and ion-lipid interactions while improved NBFIX Ca^{2+} model, by contrast, underestimates interactions between Ca^{2+} and phospholipids and overestimates the ion-ion interactions.

Establishing a new fission model: Continuous dynamin constriction cuts through membrane neck

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Membrane fission at the hourglass-shape membrane separates one membrane into two membrane compartments, which mediates many fundamental processes, such as endocytic vesicle formation, membrane-bound organelle formation, intracellular trafficking, cell and mitochondria division and viral entry. Under a common assumption that fission occurs when fission enzyme constricts the membrane neck to ~3 nm, two models have been intensely debated on the mechanisms underlying the 3-nm-neck fission. "Constriction/ratchet" model proposes simply spontaneous fission; "disassembly" model hypothesizes an energy-consuming disassembly of fission-enzyme scaffold to remove scaffold's neck-stabilizing effect, allowing the narrow neck to undergo spontaneous fission. Here we report for the most common dynamin (GTPase)-dependent fission that the models' foundational assumption is incorrect and thus the debate is invalid in live cells. We found that dynamin scaffold hydrolyses GTP (energy) to constrict Ω-shape membrane profiles' pore from hundreds of nano-meters continuously through ~3-1.3 and 1.3-0.1 nm ranges to physically force pore membrane inner leaflet scission (hemi fission), and then further constrict the hemi-fission structure to physically force membrane outer leaflet scission (hemi-to-full fission). These results suggest that pore-surrounding dynamin helix continuously surrounds and constricts the pore until not only hemi fission, but also hemi-to-full fission. This principle may broadly govern fission mediated by dynamin superfamily proteins and other fission enzymes in live cells.

3D-MINFLUX nanoscopy and membrane capacitance measurements reveal how the mouse rod ribbon builds a large active zone

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The active zone (AZ) is defined as the presynaptic territory where synaptic vesicles (SVs) are primed for release, through the coordination of SV fusion machinery with voltage dependent Ca^{2+} channels (Ca_v). Variations in structural and functional characteristics of AZs have been widely documented, and understanding how AZ proteins shape these characteristics is important. Here we describe new approaches for studying mammalian rod photoreceptors (PRs) that possess a single synaptic terminal, wherein an individual synaptic ribbon type AZ is formed. The synaptic ribbon is a specialize AZ structure that projects into the cytoplasm, and it is found only in certain sensory neurons in eyes and ears that utilize tonic glutamate release. The rod ribbon has dimensions of 35 × 200 × 1500 nm (w × h × l) and an overall ∩-shaped superstructure that tethers a couple hundred SVs. At the ribbon's base, where it contacts the plasma membrane and AZ proteins, ~60 to 90 SVs are docked and presumed to represent the readily releasable pool (RRP) of SVs. Importantly, deletion of the protein ribeye, which is uniquely expressed in the body of the ribbon, produces ribbonless rod AZ with fewer docked SVs, a smaller AZ footprint, and ribeye-ko mice have impaired visual responses (scotopic-ergs). To refine our understanding of how the ribbon shapes the biophysics of SV fusion, we made high resolution whole-cell membrane capacitance (C_m) measurements from wild type rods. This revealed that a rod ribbon releases 90 SVs (the entire RRP) in under 1 msec of depolarization, utilizes Ca_v channel facilitation to expedite release onset, and this is achieved via tight coupling (< 10 nm) between SVs and open Ca_v channels. By comparison, the ribbonless rod formed a smaller RRP of 23 SVs, and the Ca_v channels lacked facilitation as indicated by a reduced peak-I_{Ca} and a normal steady-state current. Despite these changes, the SVs maintained nano-domain coupling to Ca_v channels. The large RRP and tight coupling of SVs to Ca_v channels suggests that wt AZ release sites may reside bilaterally, on other side of the ribbon's base. To assess this, we implemented 3D-MINFLUX optical nanoscopy (at Abberior Instruments) in combination with a novel sample immobilization technique that we named Heat Assisted Rapid Dehydration (HARD), wherein a thin layer of rod terminals is transferred from fresh retinal slices directly onto glass. With a localization precision of ~5 nm, we show that bassoon−RIM2−ubMunc13-2−Ca_v1.4 complexes are formed in series along the longitudinal axis of the ribbon, and on both sides of the ribbon. In total, these results show that the rod ribbon is essential for duplicating and elongating the rod AZ to create an RRP of 90 SVs, which are under the command of Ca^{2+}-nanodomains; in addition, the ribbon promotes Ca_v channel facilitation in ways that influences the timing of release. We conclude that the large number, and the nature, of release sites affords rods the capacity to carry out precisely timed tonic release.