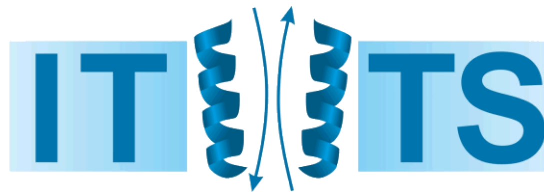

Inaugural Conference

“The Transporter Transition”

September 18 – September 21, 2018

University Clinic of Dentistry Vienna

Sensengasse 2a, 1090 Vienna, Austria

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Scientific Program

Tuesday, September 18th

09:00 - 11:00 **Registration**

11:00 - 11:15 **Opening Harald H. Sitte**

11:15 - 12:15 **Keynote lecture 1**

Chair: **Lynette Daws**
(University of Texas Health Science Center at San Antonio, USA)

11:15 - 11:20 *Introduction*

11:20 - 12:05 **Susan G. Amara**
(National Institute of Mental Health, National Institutes of Health, USA)

Regulation of neurotransmitter transporters by G-protein signaling in dopamine neurons: new insights into psychostimulant action

12:05 - 12:15 Discussion

12:15 - 13:00 **Lunch break, mounting of posters**

13:00 - 14:50 **Session 1**

The importance of organic cation transporters (OCTs, MATEs) in drug therapy

Chair: **Anne Nies**
(Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany)

13:00 - 13:10 *Introduction*

13:10 - 13:30 **Anne Nies**
(Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany)

The role of OCTs and alternative transporters in transport of and response to protein kinase inhibitors

13:30 - 13:35 Discussion

13:35 - 13:55 **Tanja Dujic**
(University of Sarajevo, Sarajevo, Bosnia and Herzegovina and University of Dundee, Dundee, Scotland, UK)

Association of OCT1 with intolerance to metformin in Type 2 diabetes

13:55 - 14:00 Discussion

14:00 - 14:20 **Mladen Tzvetkov**
(Center of Drug Absorption and Transport (C_DAT), University Medicine, Greifswald, Germany)

OCT1 pharmacogenetics in pain management: is clinical application within reach?

14:20 - 14:25 Discussion

14:25 - 14:45 **Felix P. Mayer**
(Brain Institute, Florida Atlantic University, USA)

The combination of alcohol and cocaine affects monoamine transporters of the SLC-6 family and organic cation transporter 3

14:45 - 14:50 Discussion

14:50 - 15:20 **Coffee/Tea break, refreshments**

15:20 - 17:30 Session 2

Young Scientists Session - Young ISN Neurochemistry Awardees selected from abstract

Chair: **Peter Chiba**
(Medical University Vienna, Austria)

15:20 - 15:30 *Introduction*

15:30 - 15:42 **Freja Herborg**
(University of Copenhagen, Denmark)

Loss of function variants in monoamine transportes as a risk factor for neuropsychiatric disease? – Insights from a population based case-cohort sample

15:42 - 15:45 Discussion

15:45 - 15:57 **Jenny I. Aguilar**
(Vanderbilt University, USA)

Phosphatidylinositol (4, 5)-bisphosphate coordinates functional interactions in the dopamine transporter to promote amphetamine preference

15:57 - 16:00 Discussion

16:00 - 16:12 **Louise Fets**
(Cancer Metabolism Laboratory, The Francis Crick Institute, UK)

MCT2-mediated MOG transport dictates intracellular target engagement to drive toxicity in cancer cells

16:12 - 16:15 Discussion

16:15 - 16:27 **Rita R. Fagan**
(University of Massachusetts Medical School, USA)

PKC-stimulated dopamine transporter internalization: Interdependent roles of Rin, Ack1 and transporter amino- and carboxy termini

16:27 - 16:30 Discussion

16:30 - 16:42 **Manuele Rebsamen**
(CeMM, Austria)

Identification of solute carriers involved in responses to amino acid starvation by gain-of-function genetic screens

16:42 - 16:45 Discussion

16:45 - 16:57 **Bala Krishna Prabhala**
(University of Copenhagen, Denmark)

Chloramphenicol is a substrate of prototypical proton coupled oligopeptide transporter from E. coli

16:57 - 17:00 Discussion

17:00 - 17:12 **Alyssa West**
(University of South Carolina, USA)

Serotonin in the Medial Prefrontal Cortex: How Transport is Altered in Autism Spectrum Disorder Models

17:12 - 17:15 Discussion

17:15 - 17:27 **Shreyas Bhat**
(Medical University of Vienna, Austria)

Correction of folding deficits exhibited by monoamine transporter mutants using a non-classical pharmacological approach

17:27 - 17:30 Discussion

18:30 - 21:30 Welcome Dinner, Van Swieten Hall, MedUni Vienna
Van-Swieten-Gasse 1a, 1090 Vienna

Wednesday, September 19th

08:00 - 08:45	**Registration**
08:45 - 10:35	**Session 3**
	Regulation of Dopamine Transporters: From Molecules to Behavior and Beyond
	Chair: **Haley Melikian** (University of Massachusetts Medical School, USA) Co-Chair: **Habibeh Khoshbouei** (University of Florida, USA)
08:45 - 08:55	*Introduction*
08:55 - 09:15	**Habibeh Khoshbouei** (University of Florida, USA)
	Immune regulation of dopamine transporter activity
09:15 - 09:20	Discussion
09:20 - 09:40	**Suzanne Underhill** (National Institute on Mental Health, USA)
	Mechanisms of amphetamine-mediated DAT trafficking
09:40 - 09:45	Discussion
09:45 - 10:05	**Ulrik Gether** (University of Copenhagen, Denmark)
	Nanoscale architecture of presynaptic dopamine terminals: regulation of the dopamine transporter at the single molecule level
10:05 - 10:10	Discussion
10:10 - 10:30	**Haley Melikian** (University of Massachusetts Medical School, USA)
	Dopamine transporter trafficking and regulation: Native mechanisms and behavioral consequences
10:30 - 10:35	Discussion
10:35 - 11:00	**Coffee/Tea break, refreshments**
11:10 - 12:10	**Keynote lecture 2**
	Chair: **Balazs Sarkadi** (Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary)
11:10 - 11:15	*Introduction*
11:15 - 12:00	**Satoshi Murakami** (Tokyo Institute of Technology, Japan)
	Structure and function of tripartite drug efflux transporters in Gram- negative bacteria
12:00 - 12:10	Discussion
12:10 - 13:00	**Lunch break**
12:10 - 14:15	**Postersession**

14:15 - 16:05	**Session 4**
	NCCR TransCure: interdisciplinary membrane transport research

Chair: **Bruno Stieger**
(University of Zürich, Switzerland)
Co-Chair: **Cristina Manatschal**
(University of Zürich, Switzerland)

14:15 - 14:25 *Introduction*

14:25 - 14:45 **Roch-Philippe Charles**
(University of Bern, Switzerland)

The LAT1 inhibitor JPH203 reduces growth of thyroid carcinoma in a fully immunocompetent mouse model

14:45 - 14:50 Discussion

14:50 - 15:10 **Cristina Manatschal**
(Department of Biochemistry, University of Zürich, Switzerland)

Structural and mechanistic basis of proton-coupled metal ion transport in the SLC11/NRAMP family

15:10 - 15:15 Discussion

15:15 - 15:35 **Scott Jackson**
(Institute of Molecular Biology and Biophysics, ETH Zurich, Switzerland)

Structural basis of small-molecule inhibition of human multidrug transporter ABCG2

15:35 - 15:40 Discussion

15:40 - 16:00 **Roberta de Ceglia**
(Department of Fundamental Neurosciences, University of Lausanne, Switzerland)

Exploring astrocyte-specific VGLUTs activity: a new role in controlling glutamatergic circuits relevant in Parkinson's disease and epilepsy

16:00 - 16:05 Discussion

16:05 - 16:40 **Coffee/Tea break, refreshments**

16:40 - 18:30	**Session 5**
	Innovative approaches to structural/computational/biophysical analyses of the glutamate and biogenic amine transporter families

Chair: **Susan G. Amara**
(National Institute of Mental Health, National Institutes of Health, USA)
Co-Chair: **Ivet Bahar**
(School of Medicine, University of Pittsburgh, USA)

16:40 - 16:50 *Introduction*

16:50 - 17:10 **Olga Boudker**
(Weill Cornell Medical College, USA)

Hills and valleys in the functional cycle of the thermophilic transporter GltPh

17:10 - 17:15 Discussion

17:15 - 17:35 **Claus Juul Løland**
(Department of Neuroscience, University of Copenhagen, Copenhagen, Denmark)

Substrate-induced conformational dynamics of the dopamine transporter

17:35 - 17:40 Discussion

17:40 - 18:00	**Ivet Bahar** (School of Medicine, University of Pittsburgh, USA) *Signature dynamics of LeuT family of transporters: conserved vs. specific mechanisms of motions and their functional impact*
18:00 - 18:05	Discussion
18:05 - 18:25	**Lei Shi** (National Institute on Drug Abuse - Intramural Research Program, USA) *Characterization of the dynamics in the central binding site and the extracellular vestibule of the serotonin transporter reveals new ligand discovery opportunities*
18:25 - 18:30	Discussion
20:30 - 22:30	Free evening / Jazz concert: **Benny Sharoni & Oliver Kent Trio** Porgy & Bess, Riemergasse 11, 1010 Vienna (Details: http://www.porgy.at/events/8778/)

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Thursday, September 20th

08:00 - 08:45	**Registration**
08:45 - 10:35	**Session 6** **Structure, Function and Regulation of Excitatory Amino Acid Transporters**
	Chair: **Claus Juul Løland** (Department of Neuroscience, University of Copenhagen, Copenhagen, Denmark)
08:45 - 08:55	*Introduction*
08:55 - 09:15	**Nicolas Reyes** (Molecular Mechanisms of Membrane Transport Laboratory, Institut Pasteur, Paris, France) *Ion Coupling in Human Excitatory Amino Acid Transporters*
09:15 - 09:20	Discussion
09:20 - 09:40	**Renae Ryan** (Transporter Biology Group, Sydney Medical School, University of Sydney, Australia) *The Split Personality of Glutamate Transporters: A Chloride Channel and a Transporter*
09:40 - 09:45	Discussion
09:45 - 10:05	**Baruch Kanner** (School of Medicine-IMRIC-Biochemistry and Molecular Biology, The Hebrew University of Jerusalem, Israel) *Both reentrant loops of the sodium-coupled glutamate transporters contain molecular determinants of cation selectivity*
10:05 - 10:10	Discussion
10:10 - 10:30	**Kenneth Madsen** (Department of Neuroscience, University of Copenhagen, Denmark) *Functional regulation of GLT1b by the scaffold protein PICK1*
10:30 - 10:35	Discussion
10:35 - 11:10	**Coffee/Tea break, refreshments**
11:10 - 12:10	**Keynote lecture 3**
	Chair: **Michael Freissmuth** (Medical University Vienna, Austria)
11:10 - 11:15	*Introduction*
11:15 - 12:00	**Christine Ziegler** (University Regensburg, Germany) *Impact of specific lipid-protein interactions on membrane transport*
12:00 - 12:10	Discussion
12:10 - 13:00	**Lunch break**
12:10 - 14:15	**Postersession**
14:15 - 14:45	**Business Meeting**

14:45 - 16:35	**Session 7**
	Discovery of novel cell-type specific functions of the glutamate transporter GLT-1 using a conditional knockout approach
	Chair: **Paul A. Rosenberg** (Boston Children's Hospital and Harvard Medical School, USA)
14:45 - 14:55	*Introduction*
14:55 - 15:15	**Paul A. Rosenberg** (Boston Children's Hospital and Harvard Medical School, USA)
	Multiple lines of evidence suggest neuronal GLT-1 KO mice have a synaptopathy
15:15 - 15:20	Discussion
15:20 - 15:40	**Zhou Yun** (University of Oslo, Norway)
	Axon-terminals expressing EAAT2 (GLT-1; slc1a2) are common in the forebrain and not limited to the hippocampus
15:40 - 15:45	Discussion
15:45 - 16:05	**Laura F. McNair** (University of Copenhagen, Denmark)
	The glutamate transporter GLT-1 expressed in neurons is important for glutamate homeostasis and synaptic energy metabolism
16:05 - 16:10	Discussion
16:10 - 16:30	**Selva Baltan** (Cleveland Clinik, Lerner Research Institute, Cleveland, USA)
	Oligodendrocytes Support Axon Function via Glutamate Signaling through Glutamate Transporters (GLT-1) in a Gender and Age-specific Manner
16:30 - 16:35	Discussion
16:35 - 17:10	**Coffee/Tea break, refreshments**
17:10 - 19:25	**Session 8**
	ReSOLUTE: an academic-industry IMI partnership to tackle SLCs
	Chair: **Giulio Superti-Furga** (Research Center for Molecular Medicine of the Austrian Academy of Sciences – CeMM, Austria)
17:10 - 17:20	*Introduction*
17:20 - 17:40	**Giulio Superti-Furga** (Research Center for Molecular Medicine of the Austrian Academy of Sciences – CeMM, Austria)
	ReSOLUTE: an academic-industry IMI partnership to tackle SLCs
17:40 - 17:45	Discussion
17:45 - 18:05	**Claire Steppan** (Pfizer, USA)
	SLCs and drug discovery - a pharma perspective
18:05 - 18:10	Discussion
18:10 - 18:30	**Daniel Lackner** (CeMM, Austria)
	A deorphanization pipeline for SLCs
18:30 - 18:35	Discussion

18:35 - 18:55 **Daniela Digles**
(University of Vienna, Austria)

ReSOLUTE databases and tools for the scientific community

18:55 - 19:00 Discussion

19:00 - 19:20 **Katharina L. Duerr**
(University of Oxford, UK)

Harnessing the SGC integral membrane pipeline for solute carrier protein production

19:20 - 19:25 Discussion

19:30 - 22:00 **Wine and Cheese / Postersession**

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Friday, September 21st

08:00 - 08:45	**Registration**
08:45 - 10:35	**Session 9** **Transmembrane transport of drugs and xenobiotics**
	Chair: **Douglas B. Kell** (The University of Manchester, UK)
08:45 - 08:55	*Introduction*
08:55 - 09:15	**Douglas B. Kell** (The University of Manchester, UK) *The 'real' (natural) substrates of pharmaceutical drug transporters*
09:15 - 09:20	Discussion
09:20 - 09:40	**Elizabeth Bilsland** (Unicamp, São Paulo, Brazil) *The transporter-mediated uptake and efflux of drugs across the blood-brain barrier*
09:40 - 09:45	Discussion
09:45 - 10:05	**Irina Borodina** (The Novo Nordisk Foundation Centre for Biosustainability, Danish Technical University, Denmark) *Deciphering the transport mechanisms of small molecules for improved cell factories*
10:05 - 10:10	Discussion
10:10 - 10:30	**David Dickens** (University of Liverpool, UK) *Cellular uptake of the atypical antipsychotic clozapine is a carrier-mediated process*
10:30 - 10:35	Discussion
10:35 - 11:10	**Coffee/Tea break, refreshments**
11:10 - 12:10	**Keynote lecture 4**
	Chair: **Harald Sitte** (Medical University Vienna, Austria)
11:10 - 11:15	*Introduction*
11:15 - 12:00	**Thomas Jentsch** (Leibniz-Institut fuer Molekulare Pharmakologie, Berlin, Germany) *Properties and physiological roles of VRAC/LRRC8 volume-regulated anion channels*
12:00 - 12:10	Discussion
12:10 - 13:10	**Lunch break**

13:10 - 15:00	**Session 10**
	Chloride Physiology/Homeostasis: Channels and co-transporters in Neuronal and Pancreatic B cell physiology and disease
	Chair: **Lydia Aguilar-Bryan** (Pacific Northwest Diabetes Research Institute, USA)
13:10 - 13:20	*Introduction*
13:20 - 13:40	**Claudio Rivera** (University of Helsinki, Neuroscience Center, Finnland)
	Depolarizing GABA triggers Glutamatergic sprouting in epilepsy
13:40 - 13:45	Discussion
13:45 - 14:05	**Eric Delpire** (Vanderbilt University Medical Center, Anesthesiology, Molecular Physiology and Biophysics, USA)
	GABA, K-Cl and Na-K-2Cl cotransporters in epilepsy and pain
14:05 - 14:10	Discussion
14:10 - 14:30	**Mauricio Di Fulvio** (Wright State University, School of Medicine, USA)
	Chloride transporters and channels in β-cell physiology and insulin secretion
14:30 - 14:35	Discussion
14:35 - 14:55	**Lena Eliasson** (Lund University Diabetes Centre, Sweden)
	Chloride transporters and channels in alpha-cell physiology and glucagon secretion
14:55 - 15:00	Discussion

15:00 - 15:30	**Coffee/Tea break, refreshments**

15:30 - 16:40	**Session 11**
	Young Scientists Session - Young ISN Neurochemistry Awardees selected from abstract
	Chair: **Thomas Stockner** (Medical University Vienna, Austria)
15:30 - 15:40	*Introduction*
15:40 - 15:52	**Andre Bazzone** (Nanion Technologies GmbH, Germany)
	H+ coupling and pH regulation in MFS sugar transporters: An application of SSM-based electrophysiology
15:52 - 15:55	Discussion
15:55 - 16:07	**Caroline Neumann** (Aarhus University, Denmark)
	Investigations into the binding site promiscuity of MhsT
16:07 - 16:10	Discussion
16:10 - 16:22	**Ahmad Reza Mehdipour** (Max Planck Institute of Biophysics, Germany)
	Mechanism of recognition of lipid substrates by ABC transporters
16:22 - 16:25	Discussion
16:25 - 16:37	**Dániel Szöllősi** (Medical University of Vienna, Austria)
	ABCB1 nucleotide binding domain dimerization cycle
16:37 - 16:40	Discussion

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16.40 - 16:45 **Refreshments**

16:45 - 18:35	**Session 12**
	Invertebrates as a model organism to study transporter function and dysfunction
	Chair: **Renae Ryan** (University of Sydney, Australia) Co-Chair: **Aurelio Galli** (University of Alabama Birmingham, USA)
16:45 - 16:55	*Introduction*
16:55 - 17:15	**Sonja Sucic** (Medical University of Vienna, Austria)
	Functional rescue of misfolded dopamine transporter variants by pharmacochaperoning
17:15 - 17:20	Discussion
17:20 - 17:40	**Don van Meyel** (McGill Centre for Research in Neuroscience, Canada)
	Using Drosophila to understand the function (and dysfunction) of Excitatory Amino Acid Transporters
17:40 - 17:45	Discussion
17:45 - 18:05	**Laura Bianchi** (Miller School of Medicine, University of Miami, USA)
	Glial channels and transporters that mediate excretion of ions in the microenvironment between glia and neurons shape neuronal output in C. elegans
18:05 - 18:10	Discussion
18:10 - 18:30	**David Krantz** (David Geffen School of Medicine at UCLA, USA)
	Transporter trafficking mutations and their effects on circuit function
16:30 - 18:35	Discussion
18:35 - 18:45	**Closing words/poster awards**
	Harald H. Sitte
20:00 - 23:00	**Farewell Dinner**
	Heuriger Ing. Werner Welser, Probusgasse 12, 1190 Vienna

Plenary Lectures

PL1: Regulation of neurotransmitter transporters by G-protein signaling in dopamine neurons: new insights into psychostimulant action

Susan G. Amara [1]

[1] National Institute of Mental Health, Bethesda, USA

The plasma membrane monoamine carriers serve as the initial targets for psychostimulant drugs with both therapeutic and abuse potential. However, amphetamines, amphetamine-derivatives and a variety of monoamine transporter substrates also activate signaling pathways as they accumulate intracellularly. We previously observed that once amphetamines enter neurons they can activate multiple intracellular signaling pathways. Within the cell, amphetamines activate the small GTPases, Rho and Rac1, and trigger endocytosis of the dopamine transporter and a neuronal glutamate transporter by a RhoA-dependent internalization pathway. They also increase cAMP and protein kinase A (PKA) signaling. These events depend upon the expression of an intracellular G-protein coupled trace amine receptor (TAAR1) that couples to at least two types of G-protein alpha-subunit within the cell. Using a series of subcellularly-targeted genetic FRET sensors to detect RhoA or PKA activation, we have been able to examine the subcellular membrane compartments where TAAR1 signaling events initiate. The results imply that amphetamine-like drugs not only inhibit monoamine transport and potentiate neurotransmitter action, but they also activate signaling pathways through their direct action on an intracellular GPCR target. This talk will highlight the role of TAAR1- and other GPCR-mediated signaling events in amphetamine action and will consider how they are linked to the action of a variety of drugs that modulate monoamine signaling.

PL2: **Structure and function of tripartite drug efflux transporters in Gram-negative bacteria.**

Satoshi Murakami [1]

[1] Tokyo Institute of Technology, Japan

Multidrug resistance caused by drug efflux transporter is a serious problem in antibiotic treatment of numerous bacterial infections. The envelope of Gram-negative pathogens contains unique tripartite machineries that export noxious compounds from the cell. These machineries are composed of a plasma membrane transporter and outer membrane porin that are connected by a periplasmic adaptor protein. AcrB which belong to the Resistance-Nodulation-cell Division (RND) superfamily is one of the most characterized tripartite transporter complex[1,2]. ATP-binding cassette (ABC) and major facilitator superfamily transporters can also be part of tripartite complexes, and share similar or identical components with RND transporters. The tripartite-type ABC transporter, MacB in complex with outer membrane porin, TolC and periplasmic adaptor protein, MacA, is an important efflux transporter that mediates the extrusion of macrolides, peptide toxins, virulence factors, siderophores, lipopolysaccharides and protoporphyrins. Although crystal structures of ABC transporters have been reported for both exporters and importers from various organisms, the ABC protein MacB is unrelated to AcrB, which form large trimeric complexes in the plasma membrane, raising questions about the structure and domain organization of MacB in the tripartite efflux transporter.
Recently, we solved the crystal structure of MacB at 3.4 Å resolution[3]. MacB forms a dimer in which each protomer contains a nucleotide binding domain and four transmembrane helices that protrude in the periplasm for interaction with the periplasmic adaptor protein, MacA. The MacB structure exhibits significant differences with known structures of ABC proteins, and provides a framework for further elucidation of the mechanisms of these important tripartite efflux transporters. The cryo-electron microscopy structure of MacA-MacB-TolC, the ABC tripartite assembly, solved at near-atomic resolution[4] also gives various information for understanding molecular mechanism of transport in the tripartite type ABC transporters.

[1] Murakami, S *et al*., ***Nature***, **419**, 587 (2002)
[2] Murakami, S *et al*., ***Nature***, **443**, 173 (2006)
[3] Okada, U *et al*., ***Nat. Commun***., **8**, 1336 (2017)
[4] Fitzpatrick, AWP *et al*., ***Nat. Microbiol***., **2**, 17070 (2017)

PL3: **Impact of specific lipid-protein interactions on membrane transport**

Christine Ziegler [1]

[1] University Regensburg, Germany

The functional interaction of membrane lipids with membrane transport proteins is one of the cutting-edge questions in biological science. Different mechanisms are described by which lipids affect and control membrane transport. The key in the understanding of functional lipid-protein interactions is the degree of specificity and the impact of integral physical properties of the membrane. Mechanosensitive channels are a prominent example how bulk lipids affect channel opening and closing by changes in membrane tension. In contrast transient-receptor potential (TRP) channels provide a highly specific lipid-binding pocket to regulate the selectivity filter and the transition pore. Cholesterol binding to SLC6 neurotransmitter transporter might shift the conformational equilibrium by stabilizing transporter states. Structural biology, mainly cryo-electron microscopy single particle analysis has contributed fascinating insights into lipid-mediated conformational changes in membrane proteins. However, biophysical data on the dynamics of regulatory lipid interactions is still scarce. Here, we describe different lipid interactions in the transport regulation of the LeuT-fold osmolyte transporter BetP. BetP responds to osmotically driven changes in the membrane with respect to lipid composition (specific interaction) and to membrane curvature (bulk interaction). By a combination of X-ray crystallography, single particle cryo-electron microscopy, HDX, FRET and FTIR we are able to pin point conserved lipid interaction sites within the large LeuT-fold superfamily of secondary transporters.

PL4: **Properties and physiological roles of VRAC/LRRC8 volume-regulated anion channels**

Thomas J. Jentsch [1]

[1] Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP) and Max-Delbrück-Centrum für Molekulare Medizin (MDC), Berlin

The volume-regulated anion channel VRAC is apparently ubiquitously expressed in mammalian cells. Being almost completely closed under resting conditions, it opens upon cell swelling. The resulting release of osmolytes allows the cell to regulate its volume. In addition, VRAC has man other roles (for review (1)).

While known from biophysical fingerprints for more than three decades, its molecular identity was unknown until we showed recently that it is constituted by LRRC8 heteromers (2). LRRC8A is the only essential subunit (2,3), but it needs at least one of the other subunits (LRRC8B-E) to form physiological functional channels (2). The LRRC8 subunit composition determines its inactivation kinetics at positive voltages (2,4), which depends on structures at the end of its extracellular loop (4). Importantly, LRRC8/VRAC channels not only conduct halides, but also organic compounds like taurine (2), cisplatin and other anti-cancer drugs (5), and neurotransmitters (6), with the substrate specificity depending on the subunit composition (5,6). Our structure-function analysis shows that both the second half of the first extracellular loop (4) and the extreme amino-terminus (7) of LRRC8 proteins participate in VRAC's pore. LRRC8/VRAC channels are is not only involved in cell volume regulation, but are, for instance, also involved in cancer drug resistance by a dual mechanism that includes its role in apoptosis and drug uptake (5). VRAC also enhances β-cell glucose sensing and insulin secretion by depolarizing β-cells upon glucose-induced cell swelling (8). VRAC is needed for proper development of male germ cells in a cell-autonomous manner (9). Many other important functions are likely to emerge.

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2. Voss, F. K., Ullrich, F., Münch, J., Lazarow, K., Lutter, D., Mah, N., Andrade-Navarro, M. A., von Kries, J. P., Stauber, T., and Jentsch, T. J. (2014) Identification of LRRC8 heteromers as an essential component of the volume-regulated anion channel VRAC. *Science* **344**, 634-638
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Session 1

S01-1: The role of OCTs and alternative transporters in transport of and response to protein kinase inhibitors

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Small molecule protein kinase inhibitors have emerged as backbone of cancer therapy and treatment of other diseases. In contrast to the remarkable success of the tyrosine kinase inhibitor imatinib in the improvement of outcome of patients with chronic myeloid leukemia, the degree of success of other protein kinase inhibitors is varying and most achieve only moderate survival benefits. For example, the multikinase inhibitor sorafenib is approved for the treatment of advanced clear-cell renal carcinomas and hepatocellular carcinomas, yet, patients' survival is prolonged for only several months. Like the situation when using conventional cytotoxic chemotherapy, the occurrence of drug resistance often counteracts successful treatment. In addition to somatic variation of the targeted protein kinases, insufficient drug exposure may contribute significantly to drug resistance. Here, drug uptake and efflux transporters in enterocytes, hepatocytes, proximal tubule kidney epithelial cells and not least in the cancer cells preclude sufficient intracellular protein kinase inhibitor accumulation.

While the role of ATP-binding cassette efflux transporters in *in vitro* transport and *in vivo* disposition in knockout mice has been established for many protein kinase inhibitors, their role in protein kinase inhibitor disposition and occurrence of drug resistance in humans – as assessed by pharmacogenetic association studies – is less clear. Moreover, several transporters of the solute carrier (SLC) family have been implicated in cellular uptake of protein kinase inhibitors. This presentation will describe available studies on the involvement of currently identified and investigated uptake transporters in protein kinase inhibitor transport. A special focus will be on organic cation transporter 1 (OCT1, encoded by *SLC22A1*) because there is conflicting evidence as to whether it indeed transports imatinib and sorafenib. Finally, this presentation will attempt to illustrate innovative techniques in preclinical and early clinical drug testing to advance the study of drug transport processes and of protein kinase inhibitor resistance.

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S01-2: **Association of OCT1 with intolerance to metformin in Type 2 diabetes**

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Metformin is a first-line drug for type 2 diabetes (T2D), used by over 120 million people worldwide. It has considerable benefits over other T2D therapies. However, 20-30% patients treated with metformin develops gastrointestinal (GI) side effects, and 5-10% are unable to tolerate metformin due to their severity. The mechanism for GI intolerance to metformin, and considerable inter-individual variability of these effects, is unknown. We hypothesised that reduced transport of metformin by organic cation transporter 1 (OCT1) could increase metformin concentration in the intestine, leading to increased risk of severe GI side effects and drug discontinuation.

We used detailed comprehensive prescribing data, linked to the biochemistry records and genetic information, on nearly 10,000 patients with T2D in Tayside, Scotland from 1994 onwards (GoDARTS), to identify incident users of metformin. A surrogate phenotype of metformin intolerance was defined based on discontinuation of metformin, and switch to another oral hypoglycaemic agent, within first 6 months of metformin treatment. Intolerant patients were compared with patients who were defined as tolerant based on treatment with ≥ 2000 mg of metformin for more than 6 months.

We identified older age and female sex as risk factors for metformin intolerance. Furthermore, concomitant treatment with medications, known to inhibit OCT1 activity, was associated with intolerance, as was carriage of two reduced-function OCT1 alleles compared with carriage of one or no deficient allele. Intolerance was over four times more likely to develop in individuals with two reduced-function OCT1 alleles who were treated with OCT1 inhibitors.

In the later study, we explored the association between OCT1 reduced-function variants and common metformin GI side effects in a prospectively recruited cohort of patients with newly diagnosed T2D, incident users of metformin, in Bosnia and Herzegovina. In this cohort, the number of OCT1 reduced-function alleles was significantly associated with over two-fold higher odds of common metformin-induced GI side effects. Thus, these results confirmed earlier GoDARTS findings, extending them also to the milder metformin intolerance phenotype.
Our findings have potential translational impact. If confirmed in a clinical study, avoiding drugs that interact with OCT1 could prevent serious metformin GI side effects and potential reduced efficacy associated with non-adherence and suboptimal drug dosage, as well as cessation of metformin treatment. This is particularly likely to be relevant in the 8% of the population who carry two OCT1 reduced-function alleles, where odds of GI intolerance were elevated four-fold by concomitant use of OCT1 inhibiting drugs. These results also highlight the importance of considering drug-drug interactions in pharmacogenetic studies.

S01-3: **OCT1 pharmacogenetics in pain management: is clinical application within reach?**

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Organic cation transporter 1 (OCT1/SLC22A1) is highly and almost exclusively expressed in the sinusoidal membrane of the human liver. OCT1 is highly genetically variable. Due to common genetic polymorphisms, one out of 11 Europeans and White Americans has strongly reduced or completely lack OCT1 activity. This "poor OCT1 transporter" has substantially impaired hepatic uptake of cationic and week basic drugs that may reflect in changes in the efficacy and the toxicity both of drugs acting in the liver and drugs with intensive hepatic metabolism.

In the last 6 years we learned that at least two groups of drugs used in pain management – opioids and triptans (indicated for treatment of acute migraine attacks) – may be affected by the loss of OCT1 activity due to the changes in their hepatic methabolism. This talk will summarize the *in vitro* and clinical data about the effect of OCT1 polymorphisms on the pharmacokinetics and the efficacy of the opioids morphine, codeine, and tramadol and of anti-migraine drug sumatriptan. It will present unpublished data about the effects on other opioids and discuss possible perspectives for establishing OCT1 polymorphisms as a useful tool in personalized pain management.

S01-4: **The combination of alcohol and cocaine affects monoamine transporters of the SLC-6 family and organic cation transporter 3**

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A plethora of psychoactive substances hijack the natural reward-circuitry and serve as chemical replacements for natural rewards. Cocaine, a widely abused psychostimulant, inhibits the high-affinity transporters for dopamine (DAT), serotonin (SERT) and norepinephrine (NET), thus leads to elevated concentrations of these monoamines in the extracellular space. Ethanol-intake has been associated with profound elevations in extracellular serotonin. However, ethanol does not affect SERT-function. Here we show that ethanol inhibits uptake by the organic cation transporter 3 (OCT3), an additional re-uptake mechanism for monoamines. Real-time measurements using fluorescent tracers revealed that ethanol did not perturb SERT-mediated uptake, whereas profound effects were observed on OCT3 at physiologically relevant concentrations. Similarly, ethanol inhibited uptake of [^{3}H]MPP+ into OCT3 expressing HEK293 cells, but not mock transfected cells. These findings were confirmed using *in vivo* chronoamperometry, with ethanol inhibiting 5-HT clearance in CA3 region of hippocampus in OCT3 wild-type but not in OCT3-deficient mice. These findings not only add to our general understanding of the mechanism of action of ethanol, but may also provide a molecular explanation for one of the most prevalent drug-combinations. While cocaine disrupts the function of DAT, NET and SERT, the effects of ethanol on the cocaine-insensitive OCT3 boost cocaine-induced increase in extracellular monoamines.

Session 2

S02-1: **Loss of function variants in monoamine transportes as a risk factor for neuropsychiatric disease? – Insights from a population based case-cohort sample**

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It is well established that disturbances in monoaminergic neurotransmission are involved in the pathophysiology of many neuropsychiatric diseases including ADHD, depression, autism spectrum disorder, bipolar disorder and schizophrenia. Some of the most powerful regulators of monoaminergic signaling are the monoamine transporter proteins that includes the intracellular vesicular monoamine transporter 2 (VMAT2) which sequesters monoamines into synaptic vesicles and the plasma membrane transporters of norepinephrine (NET), serotonin (SERT), and dopamine (DAT) that ensures neurotransmitter reuptake which is critical for terminating neurotransmission and for maintaining a synthesis independent supply of neurotransmitter. Despite their important function, little is known about how rare genetic variants of monoamine transporters may contribute to the genetic architecture of neuropsychiatric diseases at a populational level, but constrain metrics indicate a negative selection against loss of function variations and/or missense mutations. We proposed the hypothesis that disruptive mutations in monoamine transporters might increase the risk of neuropsychiatric disease, and that this might at least in part contribute to the negative selection against loss of function variants. To test this, we investigated the occurrence and allelic diversity of loss of function variants in a population based case-cohort sample encompassing five major mental disorders: ADHD, autism, schizophrenia, bipolar disorder, or depression.

S02-2: **Phosphatidylinositol (4, 5)-bisphosphate coordinates functional interactions in the dopamine transporter to promote amphetamine preference**

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The psychostimulant amphetamine (AMPH) mainly mediates its pharmacological and behavioral effects by increasing extracellular dopamine (DA) availability. DA homeostasis is maintained by the dopamine transporter (DAT), a presynaptic membrane protein that mediates the high-affinity reuptake of released DA from the synaptic cleft. We have previously demonstrated that AMPH induces N-term phosphorylation of the DAT, which leads to transport-mediated efflux of DA. Furthermore, we have shown that phosphatidylinositol (4, 5)-bisphosphate (PIP_2) directly interacts with the DAT and facilitates AMPH-induced DA efflux, but is not required for DA uptake. Specially, PIP_2 binds DAT through electrostatic interactions with positively charged DAT N-terminal residues. Disrupting the interactions between DAT and PIP2 or depleting PIP_2 diminishes reverse transport (efflux) of DA. Previous studies on the human serotonin transporter show that non-N-terminal PIP_2 binding sites (within the fourth intracellular loop) modulate transporter function. Here, we show that residue R443, which resides in the fourth intracellular loop of DAT, decreases DAT/PIP_2 interactions and AMPH-induced DA efflux, despite normal DA uptake. As such, this DAT variant dissociates forward transport (uptake) from reverse transport (efflux), allowing different transport functions of the DAT to be studied independently.

Using a coordinated genetic and pharmacological approach in *Drosophila melanogaster,* we translate our molecular discoveries *in vivo* to probe the physiological role for DAT efflux in motivated behavior. We demonstrate that disrupting the interaction between PIP_2 and R443disrupts AMPH preference, but not aversion.

S02-3: **MCT2-mediated MOG transport dictates intracellular target engagement to drive toxicity in cancer cells**

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α-Ketoglutarate (αKG) is a central metabolic node that plays important physiological roles in processes ranging from TCA and amino acid metabolism to epigenetics. The αKG analogue NOG (*N*-oxalylglycine) has been widely used to study αKG-dependent dioxygenases such as hydroxylases and demethylases, and its prodrug, DMOG (dimethyloxalylglycine), is a common tool in the study of HIF1α signalling. Here we demonstrate that, in buffered aqueous solutions, DMOG is rapidly hydrolysed to form the monocarboxylate methyloxaylglycine (MOG). MOG shows toxicity across a wide range of cancer types, however this toxicity is selective. Using gene expression analysis, we show that this selectivity is based on expression of the monocarboxylate transporter, MCT2, which transports MOG into cells where it is rapidly de-esterified to form NOG. In the absence of MCT2, low intracellular concentrations of NOG can interact with high affinity targets such as PHDs, allowing it to stabilise HIF in all cell lines. However, in cells expressing MCT2, NOG is able to accumulate to a level at which it can engage multiple low affinity targets. We use a range of metabolomics techniques to show that by inhibiting these targets, NOG has major effects on alpha-ketoglutarate metabolism, leading to significant impairments in TCA cycle flux with subsequent decreases in respiration and ATP production, which ultimately drive the observed toxicity. These findings demonstrate that MCT2 expression levels define intracellular concentrations of NOG, and thereby dictate the range of targets with which the compound is able to engage, illustrating the importance of understanding transporter-mediated drug-uptake and the impact it can have on compound specificity *in vivo.*

S02-4: **PKC-stimulated dopamine transporter internalization: Interdependent roles of Rin, Ack1 and transporter amino- and carboxy termini**

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Dopamine (DA) signaling is critical for movement, motivation, and reward. Synaptic DA availability is spatially and temporally limited by presynaptic DA clearance, mediated by the DA transporter (DAT). DAT coding variants in patients with DA-related neuropsychiatric disorders clearly illustrate that DAT is vital for DA homeostasis and DA-related behaviors. DAT plasma membrane expression is highly dynamic; DAT constitutively recycles to and from the plasma membrane, and protein kinase C (PKC) activation significantly stimulates DAT internalization and diminishes both DAT surface levels and function. We independently reported that both the neuronal GTPase, Rin (RIT2), and the non-receptor tyrosine kinase, Ack1 (TNK2), play requisite mechanistic roles for PKC-stimulated DAT internalization.

Importantly, although PKC-stimulated DAT internalization requires Rin and Ack1, the closely related serotonin transporter (SERT) neither interacts with Rin, nor is sensitive to Ack1 inactivation. Despite their clear role in regulated DAT endocytosis, it is currently unknown whether Rin and Ack1 are mechanistically linked to promote PKC-stimulated DAT internalization. Moreover, although Rin interacts directly with DAT, it is unknown whether DAT intracellular domains act synergistically to facilitate DAT/Rin interactions and regulated DAT internalization. Here, we used GTPase mutants and shRNA-mediated gene knockdown to test whether there is a mechanistic linkage between Rin, Ack1, and DAT, and used a chimeric protein approach to determine the DAT structural determinants required for Rin and Ack to impact DAT internalization. Preliminary results indicate that Rin is required for PKC- dependent Ack1 inactivation, placing Rin upstream of Ack1 in the PKC-stimulated DAT regulatory pathway. Ongoing experiments will examine how Rin knockdown impacts PKC-stimulated Ack1 inactivation and DAT internalization. DAT/SERT chimera studies, in which DAT N-, C- or both termini were replaced with cognate SERT domains, revealed that the DAT N-terminus, but not the C-terminus, is required for PKC-mediated internalization, but not for internalization in response to Ack1 inactivation. In contrast, both DAT N- and C-termini are required for both PKC- and Ack-dependent internalization. Pulldown studies using an extracellular bungarotoxin binding site in DAT demonstrate that PKC activation decreases the DAT/Rin association, and that PKC-dependent DAT/Rin dissociation requires the DAT N- terminus. Future FRET experiments will test whether the DAT termini are required for the DAT/Rin direct interaction. Taken together, these studies will further elucidate the interplay between two known signaling pathways that control DAT surface expression, and test whether the intracellular termini synergistically coordinate for DAT internalization in response to these signaling events.

S02-5: **Identification of solute carriers involved in responses to amino acid starvation by gain-of-function genetic screens**

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Solute carrier (SLC) transporters control fluxes of metabolites across membranes and represent thereby a critical interface between microenvironment, cellular and subcellular metabolism. Despite this critical function, a large proportion of the ~400 SLCs remains poorly studied. We and others previously identified a completely uncharacterized SLC, SLC38A9, as a lysosomal amino acid transporter that signals amino acid availability to mTORC1. In order to infer additional functional relationships between SLCs and metabolites and to identify SLCs able to sustain cell viability and proliferation under growth-limiting concentrations of essential nutrients, we performed a series of gain-of-function genetic screens using the CRISPR/Cas9-based transcriptional activation approach. Screens performed under limiting levels of arginine, using both genome-scale and SLC-focused libraries, revealed an arginine transporter (SLC7A3) as top hit, providing proof-of-principle that relevant genes can be identified with this approach. Based on our results, we screened 12 additional amino acids that are required for cell proliferation in our experimental system. This revealed candidate SLCs for multiple amino acids that will be further investigated to determine whether they mediate their effect by direct amino acid transport or indirectly through metabolic effects

S02-6: **Chloramphenicol is a substrate of prototypical proton coupled oligopeptide transporter from *E. coli***

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Chloramphenicol (Cam) is a broad-spectrum antibiotic used to combat bacterial infections in humans and animals. Cam export from bacterial cells is one of the mechanisms by which pathogens resist Cam's antibacterial effects, and several different proteins are known to facilitate this process. However, to date no report exists on any specific transport protein that facilitates Cam uptake. The proton-coupled oligopeptide transporter (POT) YdgR from *Escherichia coli* is a prototypical member of the POT family, functioning in proton-coupled uptake of di- and tripeptides. By following bacterial growth and conducting LC-MS-based assays we show here that YdgR facilitates Cam uptake. Some YdgR variants displaying reduced peptide uptake also exhibited reduced Cam uptake, indicating that peptides and Cam bind YdgR at similar regions. Homology modeling of YdgR, Cam docking, and mutational studies suggested a binding mode that resembles that of Cam binding to the multidrug resistance transporter MdfA. To our knowledge, this is the first report of Cam uptake into bacterial cells mediated by a specific transporter protein. Our findings suggest a specific bacterial transporter for drug uptake that might be targeted to promote greater antibiotic influx to increase cytoplasmic antibiotic concentration for enhanced cytotoxicity.

S02-7: **Serotonin in the Medial Prefrontal Cortex: How Transport is Altered in Autism Spectrum Disorder Models**

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Autism Spectrum Disorder (ASD) is the fastest growing developmental disability and currently affects 1 in 68 children in the US. Despite the increased prevalence, a single etiology has not been identified, but many experts agree that serotonin is a reproducable biomarker as roughly 1/3rd of individuals with ASD having high blood serotonin levels. A clear correlation between serotonin levels in the periphery and the central nervous system does not exist since serotonin cannot cross the blood brain barrier. To accurately asses serotonin neurotransmission, we use fast-scan cyclic voltammetry which allows us to make *in vivo* serotonin measurements in real time in both genetic and environmental risk factor exposure models. The genetic models used here, SHANK3 and SERT-Ala56, have previously been verified to have behavioral phenotypes associated with ASD mouse models. Likewise, to verify our model of perinatal lead exposure, we implement behavioral testing to evaluate the ASD behavioral phenotype. Preliminary results suggest the genetic models exhibit alterations in the transporters that remove serotonin from the extracellular space. The lead exposure model has demonstrated alterations in behavior as well as disruptions in serotonin neurotransmission.

S02-8: Correction of folding deficits exhibited by monoamine transporter mutants using a non-classical pharmacological approach

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Monoamine transporters (MATs) encompass membrane tethered transport proteins for serotonin (SERT), dopamine (DAT) and norepinephrine (NET). These transporters, localized primarily in pre-synaptic specializations, function in uptake and clearance of cognate neurotransmitters in Na^+-coupled manner. Transporter mediated neurotransmitter clearance, from the synaptic cleft into synaptic vesicles, effectively terminates neuronal transmission and is an integral mechanism for neurotransmitter homeostasis. Most point mutations in MATs cause ER-retention and impaired trafficking of transporters to the plasma membrane leading to loss-of-function. In DAT, clinical implications include parkinsonian-like neurodegeneration in infants and advent of dopamine transporter deficiency syndrome (DTDS). ER-retained transporters are often misfolded that can be functionally rescued by small molecules termed as 'pharmacochaperones'. These drugs assist in folding correction of mutant proteins, presumably by lowering the energy barrier imposed by the mutation. Improved folding loosens stringent quality control exerted by cytosolic and ER lumenal proteins. This partially restores MAT function (i.e. uptake of monoamines) as a consequence of improved ER export. Previous studies have identified noribogaine and bupropion as pharmacochaperones of MAT mutants. These drugs have been shown, in heterologous systems, to rescue some but not all DAT mutants (i.e., 5 of the 13 mutants reported in DTDS) warranting the need of an extended library. Both ligands display atypical binding profiles to DAT, i.e., they stabilise transporters in conformational states other than the conventional outward open one. Accordingly, we hypothesize that ligands that bind to and trap transporters in such unique conformational states can be potential pharmacochaperones. Co-incidentally, this conceptual conjecture has also been put forth in developing drugs that bind to MATs but do not exert cocaine like addictive effects (atypical inhibitors) or efflux properties of amphetamines (partial substrates). The present study aims to unify the anti-addictive and reduced psychostimulant properties of certain drugs with their previously unknown pharmacochaperoning capabilities. Using an approach that combines electrophysiology, standard biochemical-radiotracer assays and confocal microscopy, we are currently screening a library of >50 atypical inhibitors and partial substrates to MATs for their ability to rescue ER retained mutants. This proof of concept has already been verified in synthetic SERT mutants and is currently being extended to each of the documented DTDS associated DAT variants expressed in heterologous systems.

Session 3

S03-1: **Immune regulation of dopamine transporter activity**

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Parkinson's Disease (PD), primarily a disease of the central dopamine system, has unknown etiology. Recent literature suggests PD has a peripheral immune component, but how peripheral immunity and dysfunctional dopamine transmission are connected in PD remains unclear. Myeloid cells, specifically monocytes/macrophages, house their own dopamine systems and could thus be involved in peripheral-central signaling. Whether or not PD affects myeloid dopamine systems has not been studied. Here, we show that human monocyte-derived macrophages express a functional dopamine transporter (DAT) that is sensitive to immune regulation favoring reverse transport mode of activity and modulating phagocytosis, a first clue on how DAT activity links dopamine signaling and macrophages in healthy humans. In contrast, basal DAT activity and its regulation are altered in macrophages from PD patients suggesting that both peripheral and central dopamine transmission are dysregulated in PD. Our findings present macrophage DAT as a novel nexus between aberrant dopamine homeostasis and immunity in PD that may contribute to disease progression.

S03-2: **Mechanisms of amphetamine-mediated DAT trafficking**

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In dopamine neurons, the psychostimulant amphetamine drives internalization of the dopamine transporter, DAT, as well as the neuronal glutamate transporter, EAAT3. We have characterized the mechanisms of these internalization events and have found that they are dependent upon the GTPase dynamin, independent of clathrin, dependent upon intracellular calcium release as well as activation of the small GTPase, RhoA. Aspects of this system are further modulated by amphetamine-induced activation of protein kinase A (PKA). The interplay of these pathways as well as data implicating the receptors responsible for initiating these events will be presented. Further, differences between the mechanisms of DAT and EAAT3 internalization will be discussed.

S03-3: **Nanoscale architecture of presynaptic dopamine terminals: regulation of the dopamine transporter at the single molecule level**

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Dopaminergic presynaptic structures constitute the smallest functional unit in the dopamine system encompassing a unique repertoire of molecular components. Uncovering the architecture of these structures is essential to understand the molecular features underlying dopaminergic neurotransmission. By tightly controlling levels of extracellular dopamine through reuptake of released transmitter, the dopamine transporter (DAT) represents one of the key molecular components of the dopaminergic presynapse. We have recently implemented use of super-resolution, single molecule stochastic optical reconstruction microscopy (STORM) to visualize DAT distribution at subdiffraction resolution and how this is regulated in response to drugs and under different functional conditions (1). Our data revealed dynamic sequestration of DAT into cholesterol-dependent nanodomains (~70 nm mean diameter) in the plasma membrane of presynaptic varicosities and neuronal projections of cultured dopaminergic neurons. The molecular organization of DAT in nanodomains was reversibly reduced by short-term activation of NMDA-type ionotropic glutamate receptors as well as by activation nicotinic acetylcholine receptors, implicating DAT nanodomain distribution as a potential mechanism to modulate dopaminergic neurotransmission in response to excitatory input. The NMDA-induced distribution was not affected by cocaine, blockade of the dopamine D2 receptor or inhibition of NO synthase. Application of 3D dual-color dSTORM enabled visualization of the spatial distribution of DAT nanodomains relative to other molecular components of the presynapse including syntaxin1A, tyrosine hydroxylase (TH) and the vesicular monoamine transporter-2 (VMAT2). TH and VMAT-2 were found to be distinctively localized adjacent to, but not overlapping with, the DAT nanodomains. Syntaxin1A was, like DAT, distributed in nanodomains but we found little overlap with DAT nanodomains under steady state conditions. Importantly, recent experimentation has revealed a similar nanodomain localization of DAT in striatal slices as that seen in cultured neurons. Summarized, our data represent an important framework further dissecting the dynamic nanoscale architecture of dopaminergic neurotransmission and how this might change in diseases characterized by dysfunction of the dopamine system.

S03-4: **Dopamine transporter trafficking and regulation: Native mechanisms and behavioral consequences**

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Dopaminergic transmission is essential for motivation, reward, and movement, and DAergic dysfunction underlies multiple neuropsychiatric disorders, including addiction, ADHD and Parkinsonism. Following evoked DA release, extracellular DA levels are temporally and spatially limited by presynaptic uptake, mediated by the SLC6 DA transporter (DAT). DAT is also the primary target for inhibitory psychostimulants, such as cocaine and amphetamine, which require binding to DAT for their rewarding properties. Thus, mechanisms that control DAT presentation at the plasma membrane are likely to significantly impact psychostimulant actions and DAergic homeostasis. DAT is subject to constitutive and regulated endocytic trafficking, and efforts from our laboratory and others have elucidated multiple cellular mechanisms that are required for PKC-stimulated DAT endocytosis. Despite these advances, it remains unknown how endogenous signaling pathways regulate DAT in DAergic terminals. Moreover, it is unclear whether DAT regulation impacts DA signaling and DA-dependent behaviors. To probe these questions, we are leveraging chemogenetic receptors and conditional/inducible gene silencing in mice to test 1) whether Gq-coupled receptor stimulation impacts DAT trafficking in DAergic terminals, and 2) whether DAT trafficking dysfunction impacts DA-dependent physiology and behaviors. Taken together, these studies begin to shed light on the physiological relevant mechanisms regulating the DAT, and their impact on DA physiology.

Session 4

S04-1: **The LAT1 inhibitor JPH203 reduces growth of thyroid carcinoma in a fully immunocompetent mouse model**

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The L-type amino acid transporter 1 (LAT1/SLC7A5) transports essential amino acids across the plasma membrane. While LAT1 is overexpressed in a variety of human neoplasms, its expression and its role in thyroid cancer is currently unknown. Anaplastic thyroid carcinoma (ATC) is a highly aggressive malignancy for which no effective therapy exists. The purpose of this study was to explore whether the inhibition of LAT1 in ATC would affect tumor growth both in vitro and in vivo.

LAT1 was pharmacologically blocked by JPH203 in human ATC and papillary thyroid cancer (PTC) cell lines. The effects on proliferation and mTORC1 activity were addressed in vitro. A genetically engineered mouse model of ATC was used to address the effect of blocking LAT1 on tumor growth in vivo. SLC7A5 transcription was measured in patient-derived ATC samples to address the clinical relevance of the findings.

LAT1 block by JPH203 reduced proliferation and mTORC1 signaling in several human thyroid cancer cell lines. SLC7A5 transcription was found upregulated in ATC tissues derived from a genetically engineered mouse model and in ATC samples recovered from patients. JPH203 treatment induced thyroid tumor growth arrest in vivo in a fully immunocompetent mouse model of thyroid cancer. Additionally, analysis of publicly available datasets of thyroid carcinomas revealed that high LAT1 expression correlates with shorter survival of patients and reduced NIS/SLC5A5 transcription, therefore correlating potentially with radioiodine resistant tumors.

With this very first validation of LAT1 as bona fide cancer target in a carcinoma model in fully immunocompetent mouse model, our preclinical results show that LAT1 inhibition is a novel therapeutic approach in the context of thyroid cancers, and more interestingly in untreatable thyroid cancer.

S04-2: **Structural and mechanistic basis of proton-coupled metal ion transport in the SLC11/NRAMP family**

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Divalent metal ion transporters (DMTs) of the SLC11/NRAMP family transport iron and manganese across cellular membranes and are highly conserved in all kingdoms of life. Since the modulation of human DMT1 represents a promising strategy against iron overload disorders, we selected this protein family as target for TransCure. Using an interdisciplinary approach, we address questions concerning the molecular mechanism of transport and its pharmacology. Recently, we have determined the structures of the two prokaryotic DMT transporters from *Staphylococcus capitis* (ScaDMT)[1] and *Eremococcus coleocola* (EcoDMT)[2]. Interestingly, ScaDMT adopts an inward-facing state, while EcoDMT adopts an outward-facing state. These two structures define the endpoints of the transport cycle and have revealed the location of a conserved transition-metal ion binding site in the center of the transporter. Functional assays with proteoliposomes established EcoDMT as secondary active transporter that couples the symport of Mn^{2+} and protons with a K_M in the low micromolar range. Inspection of both structures revealed two protonatable residues close to the metal ion binding site that have changed their accessibility to either side of the membrane as potential candidates for proton acceptors. Mutation of one of these residues, a conserved histidine on α-helix 6b, resulted in metal ion transport that appears to be no longer coupled to protons, which implies that this residue likely plays a central role in proton transport. Our studies thus have revealed the conformational changes underlying transition-metal ion transport in the SLC11 family, have allowed important insights into the determinants of its coupling to protons and provide the basis to study the molecular mechanism of inhibition by small molecular compounds.

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S04-3: **Structural basis of small-molecule inhibition of human multidrug transporter ABCG2**

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ABCG2 is an ATP-binding cassette (ABC) transporter that protects tissues against xenobiotics, affects the pharmacokinetics of drugs and contributes to multidrug resistance. Although many inhibitors and modulators of ABCG2 have been developed, understanding their structure–activity relationship requires high-resolution structural insight. Here, we present cryo-EM structures of human ABCG2 bound to synthetic derivatives of the fumitremorgin C-related inhibitor Ko143 or the multidrug resistance modulator tariquidar. Both compounds are bound to the central, inward-facing cavity of ABCG2, blocking access for substrates and preventing conformational changes required for ATP hydrolysis. The high resolutions allowed for de novo building of the entire transporter and also revealed tightly bound phospholipids and cholesterol interacting with the lipid-exposed surface of the transmembrane domains (TMDs). Extensive chemical modifications of the Ko143 scaffold, combined with in vitro functional analyses, revealed the details of ABCG2 interactions with this family of compounds and provides a basis for the design of novel inhibitors and modulators.

S04-4: **Exploring astrocyte-specific VGLUTs activity: a new role in controlling glutamatergic circuits relevant in Parkinson's disease and epilepsy**

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Three isoforms of vesicular glutamate transporters (VGLUT1-3) are responsible for uploading glutamate into synaptic vesicles. They show non-overlapping CNS distributions and in particular, VGLUT1 and VGLUT2 are expressed in forebrain and midbrain, respectively. Although their activity is well established in neurons, their presence was demonstrated also in astrocytes by biochemical and immuno-EM techniques (*Bezzi et al. Nat Neurosci, 2004*), but their role is still unclear. To address this topic, we generated novel and exclusive conditional cell-specific transgenic mouse lines in which *Cre* activation, upon peripheral Tamoxifen administration, leads to VGLUTs ablation paralleled by fluorescent protein reporter expression. We characterized these mice by immuno- and biochemical analysis and studied astrocytic VGLUTs function in different circuits. Indeed, we first investigated the role of astrocytic VGLUT2 in midbrain circuits important for movement control and motor dysfunctions in pathology, as in Parkinson's disease. Next, we evaluated the consequences of astrocytic VGLUTs ablation in the forebrain circuitry by studying a model of acute epileptic seizures. In both models our findings suggested that astrocytic VGLUTs-dependent signaling exerts a modulatory effect on neuronal excitability. Altogether, we here unravel new and unexpected physiological roles of astrocytic VGLUTs in the control of forebrain and midbrain circuits whose perturbation is pathology-relevant. For the first time astrocytic VGLUTs are proposed as therapeutic targets, forming the basis for our collaborations within Transcure that allowed us to generate exclusive pharmacogenetic tools (VGLUT nanobodies - *Schenck et al, Biochemistry, 2017*) aimed at identify astrocytic VGLUT-expressing organelles, interactors and partners.

Session 5

S05-1: **Hills and valleys in the functional cycle of the thermophilic transporter GltPh**

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GltPh has been an excellent structural model of human glutamate transporters for years. However, functionally it is quite distinct as it is orders of magnitude slower than the human counterparts at ambient temperatures. The slow transport rate appears to be due to the infrequent transitions between the outward and inward facing states. Previously, we proposed that entry into kinetically trapped, so-called “locked”, states slowed functional dynamics of the transporter. We have characterized these states in experiments tracking conformational dynamics of GltPh by single molecule FRET microscopy. Through mutagenesis, we have explored the nature of the kinetic barriers along the transport cycle and identified a quadruple mutant, in which the key kinetic barriers have been lowered, resulting in transport rates that may approach those of human transporters.

S05-2: **Substrate-induced conformational dynamics of the dopamine transporter**

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The dopamine transporter is a member of the neurotransmitter:sodium symporters (NSSs), which are responsible for termination of neurotransmission through Na^+-driven reuptake of neurotransmitter from the extracellular space. The coordinated conformational rearrangements related to the transport mechanism have so far been poorly understood. Here we have probed the global Na^+- and dopamine-induced conformational dynamics of the wild-type *Drosophila melanogaster* dopamine transporter using hydrogen-deuterium exchange mass spectrometry. We identify Na^+- and dopamine-induced changes in specific regions of the transporter, suggesting their involvement in protein conformational transitions. Furthermore, we detect novel ligand-dependent slow cooperative fluctuations of helical stretches in several domains of the transporter, which could be a novel molecular mechanism that assists in the transporter function. Our results provide a framework for understanding the molecular mechanism underlying the function of NSSs by revealing the first detailed insight into the state-dependent conformational changes associated with the alternating access model of a eukaryotic NSS.

S05-3: **Signature dynamics of LeuT family of transporters: conserved *vs*. specific mechanisms of motions and their functional impact**

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Progress in characterizing the stuctural dynamics of biomolecules using elastic network models presents an opportunity to develop new methodologies for investigating families of proteins and identifying convergent/divergent aspects of their members' conformational dynamics relevant to their shared/differentiated mechanisms of functions. We recently developed a new method, *SignDy*, building on the widely established application programming interface, *ProDy*, for a systematic evaluation of the conformational mode spectra of proteins sharing the same fold but not necessarily similar sequences nor function, and deriving information on their signature dynamics. The analysis applied to a set of 26,899 proteins belonging to 116 protein superfamilies from CATH highlights the ability of the method to determine the evolutionarily conserved global modes of motions robustly defining functional mechanisms of action as well as the regions distinguished by distinctive dynamics which underlie the specificity of selected members. The method and corresponding online resource open the way to a dynamics-based categorization of family members providing an additional layer of information beyond that derived from sequence or structural classifications, exclusively. Application to LeuT family of proteins[1] demonstrate the conserved mechanisms of motions robustly allowing for alternating access of the substrate, as well as distinct mechanisms that differentiate family members and underlie substrate specificity and multimerization. The impact of structural dynamics on the function of specific members is illustrated, with focus on the function of dopamine transporters, including the efficiency of clearance from the synaptic and extra-synaptic regions,[2] its multimerization properties,[3] and modulation of its subcellular localization.[4]

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S05-4: **Characterization of the dynamics in the central binding site and the extracellular vestibule of the serotonin transporter reveals new ligand discovery opportunities**

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The serotonin transporter (SERT) exploits pre-existing ion gradients of Na^+, Cl^-, and K^+ to translocate serotonin from the synaptic cleft into the presynaptic neuron, and is a target for medications to treat neuropsychiatric disorders. For examples, the SERT inhibitors paroxetine and S-citalopram are among the most frequently prescribed and therapeutically effective selective serotonin reuptake inhibitors (SSRIs) in the treatment of depression and anxiety. Recent hSERT crystal structures, including the complexes of hSERT with paroxetine and S-citalopram, represent a milestone for structure-function analyses of SERT.

By analyzing the results of extensive and comparative molecular dynamics (MD) simulations of hSERT complexes, we revealed unexpected dynamics in its central ligand binding (S1) site, and unveiled a potential ambiguity in the binding orientation of paroxetine. We propose that the entropy component plays a significant role in the extraordinarily high-affinity of paroxetine at SERT. For the two S-citalopram molecules bound in the S1 site and in the extracellular vestibule (S2 site), respectively, our MD simulations suggested that their bindings are allosterically coupled by the conformation of the tenth transmembrane segment. In addition, we characterized the binding mode of a novel high-affinity S2-bound SERT inhibitor that allosterically modulates binding of S1-bound ligands.

Our resulting SERT models lay the foundation for future mechanistic studies of this important therapeutic target and provide clues for rational design of high-affinity SERT allosteric inhibitors.

Session 6

S06-1: **Ion coupling in human excitatory amino acid transporters**

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Ions fuel the transport cycle of human excitatory amino acid transporters. Our understanding of the molecular mechanisms underlying ion-coupling in human glutamate transporters has been hampered by the lack of purified protein samples for biophysical analysis. During my talk, I will present recent progress in understanding those mechanisms using thermostable variants of human EAAT1, and a combination of structural and functional techniques.

S06-2: **The Split Personality of Glutamate Transporters: a Chloride Channel and a Transporter**

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Glutamate is the predominant excitatory neurotransmitter in the mammalian central nervous system and activates a wide range of receptors to mediate a complex array of functions. To maintain efficient synaptic signaling and avoid neurotoxicity, extracellular glutamate concentrations are tightly regulated by a family of glutamate transporters termed Excitatory Amino Acid Transporter (EAATs). Altered glutamate transmission, and specifically disrupted EAAT function, has been implicated in a range of disease states including; Alzheimer's disease, episodic ataxia, epilepsy and stroke.

In addition to clearing glutamate from the extracellular space, EAATs can also function as chloride (Cl^-) channels, which contributes to ionic/osmotic balance and can affect cell excitability. The dual transporter/channel functions are mediated by distinct conformational states of the transporter and we have mapped the Cl^- permeation pathway to the interface of the transport and scaffold domain of the glutamate transporters. The EAATs use a unique mode of transport termed the 'twisting elevator' mechanism and we hypothesize that the Cl^- channel is activated during the elevator movement. Our aim is to develop a model for the dual functions of the glutamate transporters through structural and functional analysis of human (EAAT1) and prokaryotic (Glt_{Ph}) transporters. We have created a range of double cysteine mutants in cysteine-less EAAT1 and Glt_{Ph} to explore the movement of the transport domain during substrate translocation and to elucidate the conformational state/s that support an open Cl^- channel.

S06-3: Both reentrant loops of the sodium-coupled glutamate transporters contain molecular determinants of cation selectivity

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In the brain, glutamate transporters terminate excitatory neurotransmission by removing the neurotransmitter from the synapse by cotransport with three sodium ions into the surrounding cells. The binding sites of the three sodium ions have been identified by structural studies. The side-chains of the amino acid residues which ligand the sodium ions at the Na1 and Na3 sites are fully conserved in archaeal and eukaryotic glutamate transporters. The Na2 site is formed by three main-chain oxygens on the extracellular hairpin loop HP2 and one on transmembrane helix 7. In all glutamate transporters a glycine residue on HP2 is located close to the three main chain oxygens, except for the astroglial transporter GLT-1 where a serine residue occupies this position. Mutation of this residue to glycine enables transport also when sodium is replaced by lithium. Here we have studied the role of this serine/glycine switch on cation selectivity of substrate transport, using functional and simulation studies of mutants at this position in GLT-1 and the archaeal transporter Glt_{Ph}. The results indicate that the side chain oxygen of the serine residues may form a hydrogen bond with a main chain oxygen on transmembrane helix 7, resulting in an expansion of the Na2 site such that a water molecule can participate in Na2 coordination. Furthermore, we found other molecular determinants of cation selectivity on the nearby HP1 loop. Thus, subtle changes in the composition of the two hairpin loops determine the cation specificity of acidic amino acid transport

S06-4: **Functional regulation of GLT1b by the scaffold protein PICK1**

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The dominant glutamate transporter isoform in the mammalian brain, GLT1, exists as at least three splice variants, GLT1a, GLT1b, and GLT1c. GLT1b interacts with the scaffold protein PICK1 (protein interacting with kinase C1), which is implicated in glutamatergic neurotransmission via its regulatory effect on trafficking of AMPA-type glutamate receptors. The 11 extreme C-terminal residues specific for the GLT1b variant are essential for its specific interaction with the PICK1 PDZ domain, but a functional consequence of this interaction has remained unresolved. To identify a functional effect of PICK1 on GLT1a or GLT1b separately, we employed the Xenopus laevis expression system. GLT1a and GLT1b displayed similar electrophysiological properties and EC50 for glutamate. Co-expressed PICK1 localized efficiently to the plasma membrane and resulted in a 5-fold enhancement of the leak current in GLT1b-expressing oocytes with only a minor effect on [3H]glutamate uptake. Three different GLT1 substrates all caused a slow TBOA-sensitive decay in the membrane current upon prolonged application, which provides support for the leak current being mediated by GLT1b itself. Leak and glutamate-evoked currents in GLT1a-expressing oocytes were unaffected by PICK1 co-expression. PKC activation down-regulated GLT1a and GLT1b activity to a similar extent, which was not affected by co-expression of PICK1. In conclusion, PICK1 may not only affect glutamatergic neurotransmission by its regulatory effect on glutamate receptors but may also affect neuronal excitability via an increased GLT1b-mediated leak current. This may be particularly relevant in pathological conditions such as amyotrophic lateral sclerosis and cerebral hypoxia, which are associated with neuronal GLT1b up-regulation. We will further discuss how a similar functional regulation of DAT might relate to the alteration in the dopamine system in PICK1 KO mice.

Session 7

S07-1: **Multiple lines of evidence suggest neuronal GLT-1 KO mice have a synaptopathy**

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GLT-1 is the major glutamate transporter in the brain, and is expressed in astrocytes and in axon terminals in the hippocampus, cortex, and striatum. Neuronal GLT-1 accounts for only 5-10% of total brain GLT-1 protein, and its function in axon terminls is uncertain. As one approach to discovering the functions of GLT-1 expressed in presynaptic terminals, we have embarked on biased and unbiased investigations of gene expression changes in the neuronal GLT-1 KO. In a biased approach, we have investigated changes in expression of genes known to be altered in expression in Huntington's disease. In HD, synaptic dysfunction of the corticostriate synapse is well-established. Transcriptional dysregulation is a key feature of HD. We hypothesized that deletion of neuronal GLT-1, because it is expressed in axon terminals in the striatum, might produce a synaptopathy similar to that present in HD. If true, then some of the gene expression changes observed in HD might also be observed in the neuronal GLT-1 knockout. *In situ* hybridization using ^{33}P labeled oligonucleotide probes was carried out to assess localization and expression of a panel of genes known to be altered in expression in HD. We found changes in the expression of cannabinoid receptors 1 and 2, preproenkaphalin, and PDE10A in the striatum of mice in which the GLT-1 gene was inactivated in neurons by expression of synapsin-Cre, compared to wild-type littermates. These changes in expression were observed at 12 weeks of age but not at 6 weeks of age. No changes in DARPP-32, PDE1B, NGFIA, or β-actin expression were observed. In addition, we found widespread alteration in expression of the dynamin 1 gene. The changes in expression in the neuronal GLT-1 knockout of genes thought to exemplify HD transcriptional dysregulation suggest an overlap in the synaptopathy caused by HD with a synaptopathy caused by HD. These data further suggest that specific changes in expression of cannabinoid receptors, preproenkephalin, and PDE10A, considered to be the hallmark of HD transcriptional dysregulation, may be produced in HD by an abnormality of glutamate homeostasis or metabolism under the regulation of neuronal GLT-1, or a synaptic disturbance caused by that abnormality, independently of mutation in *huntingtin*.

In addition to this biased approach to assessment of gene expression alteration by neuronal GLT-1 KO, we have performed microarray, RNAseq, and metabolomic studies that reveal many changes of gene expression and function in the neuronal GLT-1 knockout, some of which are associated with synaptic development and function. Taken together, these different lines of evidence suggest signicant alteration of brain function in the neuronal GLT-1 KO with a particular impact on synaptic physiology that might be of significance for our understanding of neurodegenerative diseases.

S07-2: **Axon-terminals expressing EAAT2 (GLT-1; slc1a2) are common in the forebrain and not limited to the hippocampus**

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The excitatory amino acid transporter type 2 (EAAT2) represents the major mechanism for removal of extracellular glutamate. In the hippocampus, there is some EAAT2 in axon-terminals, whereas most of the protein is found in astroglia. The functional importance of the neuronal EAAT2 is unknown, and it is debated whether EAAT2 expressing nerve terminals are present in other parts of the brain. Here we selectively deleted the EAAT2 gene in neurons (by crossing EAAT2-flox mice with synapsin 1-Cre mice in the C57B6 background). To reduce interference from astroglial EAAT2, we measured glutamate accumulation in crude tissue homogenates. EAAT2 proteins levels were measured by immunoblotting. Although synapsin 1-Cre mediated gene deletion only reduced the forebrain tissue content of EAAT2 protein to 95.5 ± 3.4 % of wild-type (littermate) controls, the glutamate accumulation in homogenates of neocortex, hippocampus, striatum and thalamus were nevertheless diminished to, respectively, 54 ± 4, 46 ± 3, 46 ± 2 and 65 ± 7 % of controls (average ± SEM, n=3 pairs of littermates). GABA uptake was unaffected. After injection of U-^{13}C-glucose, lack of neuronal EAAT2 resulted in higher ^{13}C-labeling of glutamine and GABA in the hippocampus suggesting that neuronal EAAT2 is partly short-circuiting the glutamate-glutamine cycle in wild-type mice. Crossing synapsin 1-Cre mice with Ai9 reporter mice revealed that Cre-mediated excision occurred efficiently in hippocampus CA3, but less efficiently in other regions and hardly at all in the cerebellum.

Conclusions: (1) EAAT2 is expressed in nerve terminals in multiple brain regions. (2) The uptake catalyzed by neuronal EAAT2 plays a role in glutamate metabolism, at least in the hippocampus. (3) Synapsin 1-Cre does not delete floxed genes in all neurons, and the contribution of neuronal EAAT2 is therefore likely to be larger than revealed in the present study.

S07-3: The glutamate transporter GLT-1 expressed in neurons is important for glutamate homeostasis and synaptic energy metabolism

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It is no longer debated *if* neurons express the glutamate transporter GLT-1. Here, we seek to understand *why* GLT-1 is expressed in neurons. GLT-1 is highly expressed in astrocytes and its role here is primarily to remove glutamate after release from synapses to prevent accumulation in the extracellular space that would impede repeated signaling. In comparison to astrocytes, neurons express low levels of GLT-1, and this expression is localized almost entirely at axon terminals. Hence, we wondered whether GLT-1 might have functions in neurons distinct from the clearance function of GLT-1 in astrocytes. Broadly considered, glutamate homeostasis includes the roles of glutamate as a neurotransmitter, as a substrate for energy metabolism, and as a precursor in biosynthesis. How might glutamate taken up by GLT-1 in axon terminals be important in these various aspects of glutamate homeostasis?

We used a conditional GLT-1 knockout in which GLT-1 is deleted in neurons by expression of Cre recombinase under the synapsin 1 promoter (synGLT-1 KO) to elucidate the metabolic function(s) of GLT-1 expressed in neurons. The results reported here focus on the cerebral cortex. Our studies included *in vivo* metabolic ^{13}C-labeling studies, multiple *in vitro* experiments using brain slices, mitochondrial, and synaptosomal preparations. Synaptosomal uptake studies as well as electron microscopic immunocytochemistry in slices confirmed knockdown of GLT-1 in cerebral cortex of the synGLT-1 KO mice.

We found that the aspartate content was significantly reduced in brain extracts and in purified synaptosomes from the cerebral cortex of male synGLT-1 KO compared with control littermates. Moreover, ^{13}C-labelling of tricarboxylic acid (TCA) cycle intermediates originating from metabolism of [U-^{13}C]glutamate was significantly reduced in synGLT-1 KO synaptosomes. The decreased aspartate content was apparently the result of diminished entry of glutamate into the TCA cycle. Pyruvate recycling, a pathway necessary for full glutamate oxidation, was also reduced. ATP production was significantly increased, despite unaltered oxygen consumption, in isolated mitochondria from the synGLT-1 KO. Finally, we found increased density of mitochondria in axon terminals and perisynaptic astrocytes of synGLT-1 KO mice.

These studies provide the first extensive metabolic characterization of a mutant mouse with conditional deletion of GLT-1 in neurons to elucidate the role of neuronal GLT-1 in glutamate homeostasis. More specifically, these studies address the role of GLT-1 in axon terminals, the primary site of expression of GLT-1 in neurons. We demonstrate that abolishing the expression of the glutamate transporter GLT-1 in neurons has diverse consequences for mitochondrial function and ultrastructural distribution within terminals. Our results suggest that GLT-1 mediated uptake in axon terminals is coupled to synaptic mitochondrial function and provides glutamate for synaptic energy metabolism and biosynthetic activities.

S07-4: **Oligodendrocytes Support Axon Function via Glutamate Signaling through Glutamate Transporters (GLT-1) in a Gender and Age-specific Manner**

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Cleveland Clinic Foundation

One of the mechanisms that mediate glial cell death and axonal injury following an ischemic episode is excitotoxicity due to the reversal of the glutamate transporter GLT-1. Clinical evidence suggests stroke incidence, severity, and vulnerability varies with gender and age. Both astrocytes and oligodendrocytes express GLT-1, but their respective cell-specific contributions to ischemic WM injury and how these contributions vary with age and gender remains unknown. To address these questions we used a conditional GLT-1 knockout mouse and used Cre/lox technology to inactivate the GLT-1 gene specifically in oligodendrocytes. The conditional GLT-1 knockout mice were bred to PLP-CreERT mice, and the offspring (GLT-1flox/flox and GLT-1flox/flox; PLP-CreERT) were treated with tamoxifen (33mg/kg).

Mouse optic nerves (MONs) from young and aging control and transgenic male and female mice were obtained to record evoked compound action potentials (CAPs) at different stimulus intensities and to quantify the ability of axon function to recover after 60 min oxygen glucose deprivation (OGD). Specific deletion of oligodendrocyte GLT-1 (oGLT-1 KO) in young males results in increased CAP area of MONs. Axons are more excitable in MONs from female control mice compared to males, however, deletion of oGLT-1 abolishes the gender specific difference in excitability. Both female and male oGLT-1 KO young optic nerves demonstrated improved axon function recovery after an ischemic episode when compared to control animals. Excitability of aging axons in white matter was not different from young axons. Axons of aging male oGLT-1 KO showed improved recovery compared to age-matched control males.

Our results provide direct evidence that GLT-1 in oligodendrocytes supports axon function under control and ischemic conditions in an age- and gender-dependent manner. This support is likely to involve axon-glia glutamate signaling.

Session 8

S08-1: **ReSOLUTE: an academic-industry IMI partnership to tackle SLCs**

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RESOLUTE is a public-private partnership with 13 members from academia and industry with an overarching goal: To trigger an escalation in the appreciation and intensity of SLC research worldwide and establish SLCs as a tractable target class.

RESOLUTE is funded by a grant from the Innovative Medicines Initiative (IMI) and is resolved to create a decisive advancement in the overall tractability of the Solute Carrier class of protein transporters (SLCs) by providing practical and conceptual advances, and making its research output available openly and pre-competitively to the scientific community. We argue that the ~450 members of this class play an underappreciated role in physiology and pathology and represent a largely untapped source of new potential drug targets and thus merit the efficiency of scale that can be achieved only through systematic and coordinated efforts.

RESOLUTE's impact will transcend the funded consortium and funding period by **providing the evidence for the feasibility of turning the SLC group of proteins into accessible drug targets**. It will do so by empowering the community with reagents, research tools, protocols, and databases as well as the necessary 'social and cultural' instruments and exchange platforms. Thus, RESOLUTE will become an example of how a relatively understudied and biochemically demanding group of proteins can be 'unlocked' for research and development in a public-private partnership engaging the community. Through the coupling of an inclusive, 'open-access ethos' to the highest-possible quality of research output, RESOLUTE expects to accelerate the pace of research in the field of SLCs to the global benefit of research, also by training some of the young researchers that will lead the field in the future.

Here, we will give an overview of the RESOLUTE consortium: the basic idea, the key experimental workflow, the partners and how best to engage/involve the wider community of researchers.

S08-2: **SLCs and drug discovery: a pharma perspective**

Claire Steppan

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Healthy functioning of living cells requires strict control over the import and export of ions, metabolites and nutrients across membranes. Highly regulated transport proteins fulfill this role, and it has been estimated that ~10% of the human genome is linked membrane transport. The Solute Carrier (SLC) family is the largest class of such proteins (~400 members) and SLCs play vital roles across practically all cell-types in all tissues. The importance of SLCs in disease can be illustrated by human genetic data which suggest that roughly 50% of SLCs are associated with a human disease-related phenotype compared to a rate of only ~20% for the broader human genome. SLCs appear to be generally small molecule druggable and have given rise to a few important drug classes, such as the SSRIs for depression, and the SGLT2 inhibitors for diabetes. However, only ~2-3% of current drug targets are SLCs, Further, a recent publication analysis suggest SLCs are highly understudied with >200 SLCs having less than 15 publications where the target is mentioned. These data suggest SLCs are heavily underexploited as drug targets. The ReSolute consortium was established to unlock the therapeutic potential within the SLC gene-family by overcoming many of the technical barriers that have hindered their study and prevented their exploitation as drug targets This talk will focus on drivers for unlocking our knowledge on SLC transporters as therapeutics targets and highlight strategies utilized to identify small molecules that modulate SLC transporters.

S08-3: **A deorphanization pipeline for SLCs**

Daniel Lackner

Research Center for Molecular Medicine of the Austrian Academy of Sciences – CeMM

The RESOLUTE consortium consisting of 13 partners from academia and industry has one major goal: to provide the tools and data to increase research and knowledge on the biology of solute carriers (SLCs) and to establish SLCs as a tractable target class.

The large-scale data sets generated by RESOLUTE should help to deorphanize many of the SLCs for which no substrate or function is yet described. Here, I will describe the pipeline that will allow us to achieve this, from the conception to the presentation of initial data. I will give an overview of the cell line engineering process for the generation of knock-out as well as over-expression cell line models that will serve as the powerful base for future downstream experiments. I will introduce the omics approaches (proteomics, metabolomics, ionomics, transcriptomics) as well as the genetic interaction experiments planned that will allow for a "global" deorphanization of SLCs. I will furthermore discuss the more specific transporter assays and the generation of protein reagents for a selected subgroup of focused SLCs. Finally, I will give a first glimpse on how these data will be integrated and will be accessible for the broader scientific community.

S08-4: **ReSOLUTE databases and tools for the scientific community**

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The ambitious plan of the ReSOLUTE project to intensify the research on solute carriers (SLCs) by systematically generating and developing amongst others cell lines and assays for a high number of SLCs will lead to a large amount of data. To share this data both within the consortium and with the scientific community, a data- and knowledgebase will be implemented which can be accessed via a web portal. This web portal will also contain interfaces to the ReSOLUTE reagents portal and an SLC human genetics portal. Following the open access ethos of the ReSOLUTE project, the web portal will provide public access to verified data, with the plan to have all generated data available for the scientific community by the end of the project.

Using the knowledgebase and integrating data from public databases, data mining tools will aid the deorphanisation strategies and will provide input for the prioritization of SLCs. As example tool, a KNIME workflow which collects data on proteins from different public databases will be presented. This workflow was used to annotate SLCs with available information (e.g. pharmacology information from ChEMBL, patent counts from SureChEMBL, cellular pathways from Wikipathways, and diseases from DisGeNet) to help prioritize which proteins will be investigated first in the course of the ReSOLUTE project

S08-5: **Harnessing the SGC integral membrane protein pipeline for solute carrier protein production**

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The solute carrier (SLC) superfamily is the largest family of transport-related membrane proteins, encompassing approximately 400 human members. SLCs have important roles in many fundamental physiological processes, and a quarter of all SLC genes have been associated with human diseases. SLCs are not only targeted by a number of approved drugs, but are also key players for the effective delivery of therapeutics, such as the transport of CNS-targeted drugs across the blood-brain barrier. Here we describe how we utilize and improve the existing pipeline for human membrane protein production at the Structural Genomics Consortium (SGC) to facilitate a family-wide effort toward the purification of solute carrier proteins for cell-free assays and the production of high affinity binders.

Session 9

S09-1: **The 'real' (natural) substrates of pharmaceutical drug transporters.**
Consensus rank orderings of molecular fingerprints illustrate the similarities between marketed drugs and small endogenous human metabolites, but highlight exogenous natural products as the most important 'natural' drug transporter substrates.

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Transporters have important roles in both cellular drug uptake [1] and biotechnology [2]. Often their 'natural' substrates are unknown. We compare several molecular fingerprint encodings for marketed, small molecule drugs, and assess how their rank order varies with the fingerprint in terms of the Tanimoto similarity to the most similar endogenous human metabolite as taken from Recon2. For the great majority of drugs, the rank order varies very greatly depending on the encoding used, and also somewhat when the Tanimoto similarity (TS) is replaced by the Tversky similarity [3-7]. However, for a subset of such drugs, amounting to some 10% of the set and a Tanimoto similarity of ~0.8 or greater, the similarity coefficient is relatively robust to the encoding used. This leads to a metric that, while arbitrary, suggests that a Tanimoto similarity of 0.75-0.8 or greater genuinely does imply a considerable structural similarity of two molecules in the drug-endogenite space. Although comparatively few (<10% of) marketed drugs are, in this sense, robustly similar to an endogenite, there is often at least one encoding with which they are genuinely similar (e.g. TS > 0.75). This is referred to as the Take Your Pick Improved Cheminformatic Analytical Likeness or TYPICAL encoding, and on this basis some 66% of drugs are within a TS of 0.75 to an endogenite [8].

We next explicitly recognise that natural evolution will have selected for the ability to transport dietary substances, including plant, animal and microbial 'secondary' metabolites, that are of benefit to the host. These should also be explored in terms of their closeness to marketed drugs. We thus compared the TS of marketed drugs with the contents of various databases of natural products. When this is done, we find that some 80% of marketed drugs are within a TS of 0.7 to a natural product, even using just the MACCS encoding. For patterned and TYPICAL encodings, 80% and 98% of drugs are within a TS of 0.8 to (an endogenite or) an exogenous natural product. This implies strongly that it is these exogeneous (dietary and medicinal) natural products that are more to be seen as the 'natural' substrates of drug transporters (as is recognised, for instance, for the solute carrier SLC22A4 and ergothioneine [9; 10]). This novel analysis [8] casts an entirely different light on the kinds of natural molecules that are to be seen as most like marketed drugs, and hence potential transporter substrates, and further suggests that a renewed exploitation of natural products space [11] as drug scaffolds (or indeed inhibitors) would be amply rewarded. The heterogeneous uptake is due to the enormous heterogeneity of transporter expression [12].

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S09-2: **The transporter-mediated uptake and efflux of drugs across the blood-brain barrier**

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Drugs directed to the central nervous system frequently fail in clinical trials because of their poor transport into the brain. This is due to their inefficient import though the human blood-brain barrier (BBB). Hence, the development of methodologies for the study of how these compounds cross the membrane is essential for the development of drugs that efficiently target the CNS.

In our group, we use the yeast *Saccharomyces cerevisiae* as a platform for studying the transport of chemicals into and out of cells. We have constructed yeast library with deletions of pairs of genes expressing of each of the 121 non-essential plasma membrane transporters, and use these in high-throughput screens to identify groups of proteins with overlapping substrate specificities as well as to define chemical groups preferentially using that particular import route. Our rationale is to identify how drugs may cross the yeast plasma membrane and translate these findings to the orthologue transporters of the human BBB.

We are also performing high-content screens to identify novel compounds capable of inhibiting the aggregation of human FUS, TAU and α-synuclein. Once compounds capable of inhibiting human protein aggregation are selected, we identify which carriers are responsible for their entry into yeast cells. This knowledge is used to suggest putative human BBB transporters that can potentially promote the uptake of anti-aggregation compounds.

The candidate human BBB transporters are then be expressed in yeast strains where the native ergosterol synthesis pathway has been replaced for a synthetic human cholesterol synthesis pathway, to optimize the membrane environment for the human transporters. This allows us to identify BBB transporters that could potentially promote the uptake of novel compounds to treat ALS, Alzheimer's and Parkinson's diseases.

S09-3: **Deciphering the transport mechanisms of small molecules for improved cell factories**

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A shift towards bio-based production of chemicals and fuels will significantly reduce our dependency on oil and gas and lead to a more environmentally friendly economic growth. The yeast Saccharomyces cerevisiae is the cell factory of choice for production of many bulk and fine chemicals and fuels. Secretion of products into fermentation broth presents many advantages for the fermentation process, such as reduction of product feedback inhibition and degradation, increased cell fitness, no restrains on the titer by the biomass concentration. Additionally, the recovery of a given product from the broth becomes much cheaper and technically simpler, because there is no need to break the cells to extract the product and higher purity is obtained.

In this presentation, I will discuss how the transport of small molecules, such as p-coumaric acid, resveratrol, and dicarboxylic acids, can be engineered to improve the yeast cell factories.

S09-4: **Cellular uptake of the atypical antipsychotic clozapine is a carrier-mediated process**

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The weak base antipsychotic clozapine is the most effective medication for treating refractory schizophrenia. The brain-to-plasma concentration of unbound clozapine is greater than unity indicating transporter-mediated uptake, which has been insufficiently studied. This is important because it could have significant impact on clozapine's efficacy, drug-drug interaction and safety profile. A major limitation of clozapine's use is the risk of clozapine induced agranulocytosis/ granulocytopenia (CIAG), which is a rare but severe haematological adverse drug reaction.

We firstly studied the uptake of clozapine into human brain endothelial cells (hCMEC/D3). Clozapine uptake into cells was consistent with a carrier-mediated process, which was time dependent and saturable (Vmax=3299 pmol/million cells/min, Km=35.9 µM). The chemical inhibitors lamotrigine, quetiapine, olanzapine, prazosin, verapamil, indatraline and chlorpromazine reduced the uptake of clozapine by up to 95%. This could in part explain the in vivo interactions observed in rodents or humans for these compounds. An extensive set of studies utilising transporter-overexpressing cell lines and siRNA-mediated transporter knockdown in hCMEC/D3 cells, showed that clozapine was not a substrate of OCT1 (SLC22A1), OCT3 (SLC22A3), OCTN1 (SLC22A4), OCTN2 (SLC22A5), ENT1 (SLC29A1), ENT2 (SLC29A2), and ENT4/PMAT (SLC29A4).

In a recent genome-wide analysis the hepatic uptake transporters SLCO1B1 (OATP1B1) and SLCO1B3 (OATP1B3) were identified as additional candidate transporters. We therefore also investigated clozapine transport into OATP1B-transfected cells and found that clozapine was neither a substrate nor an inhibitor of OATP1B1 and OATP1B3.

In summary, we have identified a carrier-mediated process for clozapine uptake into brain, which may be partly responsible for clozapine's high unbound accumulation in the brain and its drug-drug interaction profile. Cellular clozapine uptake is independent from currently known drug transporters and thus, molecular identification of the clozapine transporter will help to understand clozapine's efficacy and safety profile.

Dickens, D., S. Radisch, G. N. Chiduza, A. Giannoudis, M. J. Cross, H. Malik, E. Schaeffeler, R. L. Sison-Young, E. L. Wilkinson, C. E. Goldring, M. Schwab, M. Pirmohamed and A. T. Nies (2018). "Cellular uptake of the atypical antipsychotic clozapine is a carrier-mediated process." Mol Pharm. In Press.

Session 10

S10-1: **Depolarising GABA triggers Glutamatergic sprouting in epilepsy**

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COI: International patent application (EPI15306384)

Remodelling of glutamatergic neuronal networks is one of the major pathophysiological processes observed in epileptic tissue and post-traumatic brain injuries. Understanding the molecular mechanisms involved is of particular importance since this sort of structural plasticity creates a powerful hyperexcitable cerebral focus that drives recurrent disabling seizures. Besides the rewiring of excitatory circuit, an abnormal depolarizing GABAergic drive has been hypothesized to participate in epileptogenic processes. This includes the deregulation of functional expression of the neuronal specific K^+-Cl^- co-transporter KCC2 and the Na^+-K^+-$2Cl^-$ co-transporter NKCC1. We have also previously shown that depolarizing GABAergic transmission triggers the up-regulation of the pan-neurotrophin receptor, $p75^{NTR}$. We have now tested the hypothesis that the early alteration of Cl^- homeostasis following status epilepticus (SE) is a precipitating event that triggers recurrent mossy fibre sprouting *via* the activation of $p75^{NTR}$. We also examined a novel therapeutic strategy based on the transient blockade of Na^+-K^+-$2Cl^-$ co-transporter NKCC1 early after SE to reduce ectopic sprouting and recurrent seizures in the chronic phase (i.e. several months after SE). The findings from this study define promising and novel targets to constrain reactive glutamatergic network rewiring in adult epilepsy

S10-2: **GABA, K-Cl and Na-K-2Cl cotransporters in epilepsy and pain**

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Due to the large electronegativity of the neuronal membrane, the resting Cl^- concentration in neurons is very low. The Cl^- concentration, however, is never at its resting value, but lower than equilibrium in central neurons and higher than equilibrium in peripheral sensory neurons. The Cl^- gradient is generated by secondary active cation-chloride cotransporters as the outward K-Cl cotransporter, KCC2, and the inward Na-K-2Cl cotransporter, NKCC1. The Cl^- gradient defines the direction of GABA-mediated Cl^- currents: hyperpolarizing in central neurons and depolarizing in sensory neurons. Disruption of these secondary active "Cl^- pumps" affects neuronal excitability, leading to CNS hyper-excitability and epilepsy as well as changes in pain perception. Several mutations have been found in human *SLC12A5*, the gene encoding KCC2. These mutations lead to several forms of epilepsy. Recently, we described a patient with a *de novo* mutation in *SLC12A2*, the gene encoding NKCC1. Among other symptoms, the patient experienced seizure activity, photophobia, and increased pain perception. Fortunately, this patient does not experience any sensorineural deafness or cognitive deficits. Following this first case report, additional mutations were found in NKCC1 associated with progressive hearing loss, mental retardation, and autistic behavior. These novel observations indicate that the SLC12A2 gene is haploinsufficient and might play a critical role in the development and maturation of the nervous system.

S10-3: **Chloride transporters and channels in β-cell physiology and insulin secretion**

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Pancreatic β-cells secrete insulin in a very precise manner by a process involving a remarkably wide variety of factors including neurotransmitters (GABA, dopamine, glycine, nor-epinephrine/epinephrine), hormones (glucagon, somatostatin, growth hormone), incretins (GLP-1 and GIP) and others. The ability of β-cells to depolarize, regulate $[Ca^{2+}]_i$ and secrete insulin, even in the absence of functional K_{ATP} channels strongly suggests the presence of additional ionic cascades of events within the process of stimulus-secretion coupling. The purpose of this talk is to re-introduce a long-relegated and largely ignored topic on the regulation of the intracellular chloride concentration ($[Cl^-]_i$) by a complex interplay between Cl^- transporters and channels in β-cells and their involvement in glucose stimulated insulin secretion. It is important to keep in mind that, in the last decade, the molecular identification and functional characterization of diverse Cl^- transporters and channels in β-cells have added an extra layer of complexity to the extraordinarily intricate secretory response. In spite of the complexity in signaling pathways, glucose-induced insulin secretion by β-cells is commonly condensed into a very simple consensus model, remarkably similar, although not identical, to the well-characterized depolarization-secretion coupling observed in some neurons, which depends on K_{ATP} cannels. Like immature neurons and nociceptors, which depolarize in response to GABA and other activators of Cl^- channels, the Cl^- loaders and extruders expressed in β-cells are believed to set and keep $[Cl^-]_i$ above equilibrium thus stablishing and outwardly directed gradient. This makes possible the depolarizing efflux of Cl^- upon opening of anion channels, such as $GABA_A$, *Cftr*, *Ano1*, volume regulated anion channels (VRAC), and others, all contributing to insulin secretion in response to glucose. We have just begun the characterization of Cl^- transporters and channels in β-cells and found several surprises, which altogether are improving our understanding of the anionic regulation of insulin secretion.

S10-4: **Chloride transporters and channels in alpha-cell physiology and glucagon secretion**

Lena Eliasson

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Alpha cells of the pancreatic islet produce glucagon, the main 'counter-regulatory' hormone in the body. This hormone is released during fasting and extensive exercise to protect against hypoglycemia and restore normoglycemia. Currently, we have a poor understanding of the physiological regulation of glucagon secretion, where both intrinsic and paracrine mechanisms have been suggested to regulate this process. Alpha cell dysfunction is present in patients with diabetes mellitus and in patients with cystic fibrosis (CF), whose most common morbidity is the development of Diabetes (CFRD). CF is caused by mutations in the ABCC9 transporter and Cl− channel, cystic fibrosis transmembrane conductance regulator (CFTR). Indeed, it remains poorly defined or unknown whether CFTR as well as the expected machinery involved in [Cl–]i regulation i.e., anion channels and Cl– cotransporters are present in the islet endocrine cells. Accordingly, the potential role they may play in the regulation of glucagon secretion has yet to be defined. Recently we have shown that both human and mouse alpha cells express CFTR-currents and that glucagon secretion is accentuated upon CFTR-inhibition in human islets. CFTR is suggested to mainly regulate the membrane potential through an intrinsic alpha cell effect, as supported by a mathematical model of alpha cell electrophysiology. In conclusion, CFTR channels are present in alpha cells and act as important negative regulators of cAMP enhanced glucagon secretion through effects on alpha cell membrane potential and may be partially responsible for the loss-of-function mutations that contribute to CFRD.

Session 11

S11-1: **H+ coupling and pH regulation in MFS sugar transporters: An application of SSM-based electrophysiology**

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In contrast to conventional electrophysiology, Solid-supported-membrane (SSM) -beased electrophysiology is designed for the measurements of electrogenic transporters (symporters, exchangers and uniporters) and pumps. Usually these proteins have low turnover rates compared to ion channels. SSM-based electrophysiology compensates for that with a large sensor size which allows for the measurement of up to 10^9 transporters at the same time.

We used SSM-based electrophysiology to characterize proton-coupled sugar transporters of the major facilitator superfamily (MFS). Because a number of structures of MFS sugar symporters have been solved recently, molecular insight into the transport mechanism is possible from detailed functional analysis. We present here a comparative electrophysiological study of the lactose permease ($LacY_{Ec}$), the fucose permease ($FucP_{Ec}$), the xylose permease ($XylE_{Ec}$) and the glucose permease ($GlcP_{Se}$), which reveals common mechanistic principles and differences.

A comparison of the pH dependence of symport at symmetrical pH exhibits broad bell-shaped pH profiles and a decrease at extremely alkaline and at acidic to neutral pH. Experimental evidence suggests that the alkaline pK_{app} is due to H+ depletion at the protonation site, while the acidic pK_{app} is due to inhibition of deprotonation. Since previous studies suggest that a single carboxyl group in LacY (Glu325) may be the only side chain directly involved in H+ translocation and a carboxyl side chain with similar properties has been identified in FucP (Asp46) and XylE (Asp27), the results imply that the pK of this residue is switched during H+/sugar symport in all three symporters.

$GlcP_{Se}$ and $XylE_{Ec}$ are homologs of the human GLUT sugar transporters. Both are equipped with a conserved proton-binding site arguing for an electrogenic transport mode. However, the electrophysiological analysis of $GlcP_{Se}$ reveals important differences between the two GLUT homologs. $GlcP_{Se}$, unlike $XylE_{Ec}$, does not perform steady-state electrogenic transport at symmetrical pH conditions. Furthermore, when a pH gradient is applied, partially uncoupled transport modes can be generated. In contrast to other bacterial sugar transporters analyzed so far, in $GlcP_{Se}$ sugar binding, translocation and release are also accomplished by the deprotonated transporter. Based on these experimental results, we conclude that coupling of sugar and H+ transport is incomplete in $GlcP_{Se}$. This phenomenon can be described by a general 8-state kinetic model.

Results are partly published in the following two papers:

pH Regulation of Electrogenic Sugar/H+ Symport in MFS Sugar Permeases.
10.1371/journal.pone.0156392

A Loose Relationship: Incomplete H^+/Sugar Coupling in the MFS Sugar Transporter GlcP.
10.1016/j.bpj.2017.09.038.

S11-2: **Investigations into the binding site promiscuity of MhsT**

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Neurotransmitter:sodium symporters (NSSs) are secondary transporters, placed on the presynaptic cell of the synapse. They ensure uptake of neurotransmitter molecules from the synaptic cleft, using energy released from the downward movement of Na^+ along its electrochemical gradient. Therefore, NSSs have an important role in controlling impulse signaling in neurons and a number of diseases are related to dysfunctions of neurotransmitter transporters. Insight into transporter structure and function has been obtained from structures of bacterial and eukaryotic members.

MhsT, a bacterial homologue of eukaryotic neurotransmitter:sodium symporters and amino acid transporters, functions as a secondary transporter of hydrophobic amino acids in *Bacillus halodurans*[1]. The goal of this project is to study the structural basis of the binding site promiscuity of MhsT. Crystal structures of MhsT in complex with Phe (2.25 Å), Tyr (2.3Å), a tyrosine orthologue 4-F-Phe (2.26 Å), Val (2.6Å), Leu (2.35Å) and Ile (3.10Å) have been determined from crystals grown using the HiLiDe method [2]. Like the previously determined MhsT-Trp structure [3], the protein is in an occluded inward- facing conformation for the six substrates. This conformation of the protein reflects the state just before the opening of the transporter to the intracellular side, and gives insight into how sodium drives this process [3]. The binding sites, however, while similar have some important differences that mark the bound substrate.

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S11-3: **Mechanism of recognition of lipid substrates by ABC transporters**

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Moving lipids between two leaflets of biomembranes is a vital process for the homeostasis in cellular life. Accumulating data indicate lipid as a major substrate of ABC transporters that link these transporters to different physiological pathways. ABC transporters involved in this process provide the starting point to understand the mechanism of lipid recognition and transport in these proteins. Given the significance of the role of lipids as possible primary substrates of ABC transporters, using different computational methods we have been able to look at the process of possible mechanism of lipid recognition and transport. Our results show that specific tight interaction of lipids with distinct hotspots on the protein surface is exploited as a recognition step and helps these proteins to recruit lipids into the binding site and flip them to the other side of the membrane.

S11-4: **ABCB1 nucleotide binding domain dimerization cycle**

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P-glycoprotein (a.k.a. MDR1 or ABCB1) is expressed at cellular barriers and causes resistance against chemotherapy if expressed in cancer cells. The pseudo-symmetric ABCB1 consists of two transmembrane domains (TMD) that bind and transport the substrate, and of two nucleotide binding domains (NBDs) that energize the transport by ATP binding and hydrolysis. It is accepted that dimerization is triggered by ATP binding, which switches the protein to the outward facing conformation and leads to cargo release to the extracellular side. Hydrolysis of ATP to ADP and inorganic phosphate eventually resets ABCB1 and induces the conformation of the inward conformation, which is of high affinity for substrate.

We study the energetics of NBD dimer formation and the role of nucleotides using biased molecular dynamics simulations of isolated NBDs. Potential of mean force profiles show that the apo configuration weakly favors the dimeric conformation, while a deep energy minimum (~ -42 kJ/mol) for the dimeric state is reached in the presence of ATP. Conserved motif interaction network analyses revealed that ATP stabilizes the NBD dimer by forming strong attractive interactions with both domains. These forces are multilayered and consist of electrostatic, hydrophobic and water mediated interactions between the nucleotide and the NBDs. ATP hydrolysis to ADP and HPO4 changes the potential and the forces. The free energy hypersurface reveals that the closed dimer became an unstable high energy state, therefore favoring the transition to an open conformation.

Our data show that ATP binding and its hydrolysis serve as sequential energy input, promoting respectively closure and the opening of the NBD dimer. The transport cycle of ABCB1 can be followed through the changes in the energy hypersurface.

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Session 12

S12-1: **Functional rescue of misfolded dopamine transporter variants by pharmacochaperoning**

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Dopamine dysfunction is linked to many psychiatric diseases, from schizophrenia and affective disorder to ADHD and parkinsonism. Recent reports in the literature revealed that point mutations in the gene encoding the dopamine transporter (hDAT, SLC6A3) cause a syndrome of infantile/juvenile dystonia and parkinsonism in children. Many of these mutations trigger folding defects in the transporter. We examined the molecular mechanisms underlying DAT folding, by providing a proof-of-principle for the rescue of folding-deficient DAT variants by means of pharmacochaperoning. We examined a Drosophila melanogaster mutant dDAT-G108Q, which leads to a sleepless phenotype in flies (1). Molecular dynamics simulations suggested an unstable structure of dDAT-G108Q, consistent with a protein folding defect. This conjecture was verified: heterologously expressed dDAT-G108Q and the human equivalent hDAT-G140Q were retained in the endoplasmic reticulum in a complex with endogenous folding sensors (calnexin and HSP70-1A). Incubation of HEK293 cells with noribogaine (i.e. an inhibitor selective for the inward-facing DAT conformation) and/or pifithrin-μ (an HSP70 inhibitor) restored the folding and function of dDAT-G108Q and hDAT-G140Q. The mutated versions of DAT were confined to the cell bodies of dopaminergic neurons in the fly brain and failed to reach the axonal compartments. Axonal delivery was restored and sleep time increased to normal length (from 300 to 1000 min/d), when dDAT-G108Q expressing flies were treated with noribogaine and/or pifithrin-μ (1). Notably, we further showed that several clinically-relevant human DAT (hDAT) mutations causing infantile dystonia/parkinsonism can be functionally remedied by pharmacochaperoning (2). Of the 13 misfolded hDAT variants investigated in our study, 3 (hDAT-V158F, hDAT-G327R, and hDAT-L368Q) responded to noribogaine and pifithrin-μ treatment, restoring their cell surface expression and uptake activity in HEK293 cells. We also exploited the power of targeted transgene expression of mutant hDATs in Drosophila to explore whether folding deficits can be pharmacologically remedied in an intact organism. Noribogaine or pifithrin-μ treatment sustained hDAT delivery to the presynaptic terminals of dopaminergic neurons and restored sleep to normal length in DAT-deficient (fumin) Drosophila lines expressing hDAT-V158F or hDAT-G327R. On the contrary, expression of hDAT-L368Q set off a developmental lethality, insinuating a toxic action, not alleviated by pharmacochaperoning (2). Moreover, our latest data provide evidence for other DAT ligands (partial substrates such as naphthylpropane-2-amines of the phenethylamine library (PAL)) acting as effective pharmacochaperones of misfolded DAT variants that failed to respond to noribogaine or pifithrin-μ. This implies a necessity for a rational search for pharmacochaperones, i.e. screening and design of compounds which may be beneficial in functionally correcting individual clinically relevant folding-deficient transporters. Taken together, rescuing misfolded DATs is therapeutically relevant and may provide promising therapeutic strategies in the treatment of pathological disorders triggered by folding defects of other SLC6 transporters, e.g. folding-deficient mutations in hCRT-1 and hGAT-1, which give rise to severe mental retardation and epilepsy, respectively.

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S12-2: **Using Drosophila to understand the function (and dysfunction) of Excitatory Amino Acid Transporters**

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Excitatory Amino Acid Transporters (EAATs) are most noted for transport of glutamate into glia, primarily astrocytes, to control neurotransmission in the brain, and to prevent neuronal cell death. EAATs are also chloride (Cl^-) channels. Loss of EAAT function is observed in epilepsy, stroke, and neurodegenerative diseases. Furthermore, EAAT1 mutations cause episodic ataxia. We use Drosophila to model EAAT dysfunction in neurological diseases, and to discover new mechanisms of EAAT function in regulating conserved behaviors. In a model for an EAAT1 mutation found in a patient with episodic ataxia, we investigated the consequences for glia and motor behavior and provided evidence linking an EAAT Cl^- channel and disease. We will discuss our latest efforts to 1) explore this mechanism for episodic ataxia, 2) interrogate EAAT Cl^- channels in normal brain function and 3) describe new mechanisms by which EAATs, and the glial cells that express them, influence motor and sleep behaviors.

12-3: **Glial channels and transporters that mediate excretion of ions in the microenvironment between glia and neurons shape neuronal output in C. elegans**

Laura Bianchi

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Isolated microenvironments, such as the tripartite synapse, exist throughout the nervous system. The regulation of ions in these microenvironments is mediated mainly by glia, but the molecular mechanisms of ion regulation and the effects on neuronal output are poorly understood. We published that DEG/ENaC Na+ channels expressed in glia control neuronal Ca2+ transients and animal behavior in response to sensory stimuli, including nose touch. We hypothesized that DEG/ENaC channels exert this type of regulation via effects on the ionic composition of the microenvironment between glia and neurons. To test this model, we used RNAi to knock-down the 5 α-subunits of the Na+/K+-ATPase in OLQ socket glial cells and asked whether nose touch response, which is mediated by OLQ neurons, was altered. We found that Na+/K+ pump α-subunits *eat-6* and *catp-1*, expected to contribute to regulation of Na+ and K+ concentrations inside and outside the cell, are required in OLQ socket glia for nose touch response. By supplementation of the nematode growth media with different osmolites, we found that glucose rescues *eat-6* and *catp-1* mutant phenotype. Experiments are in progress to establish whether the effect of glucose is mediated by regulation of transcription or translation and whether ATP is required. Our work establishes the critical role of glial channels and transporters in regulating neuronal output and behavior via regulation of the concentration of ions and other solutes. Furthermore, our data suggest that glial transporter systems are regulated by glucose availability.

S12-4: **Transporter trafficking mutations and their effects on circuit function**

David Krantz

David Geffen School of Medicine at UCLA, USA

Vesicular Monoamine Transporters are responsible for the storage of aminergic neurotransmitters in both mammals and the model organism Drosophila melanogaster. We we using the Drosophila Vesicular Monoamine Transporter (DVMAT) to explore the effects of trafficking mutants on aminergic circuit function, focussing on simple circuits amenable detailed molecular genetic analyses that would be more difficult in mammalian systems. We will report on the development of the oviposition (egg-laying) circuit as a tool to study DVMAT trafficking mutations that disrupt localization to specific types of secretory vesicles.

Poster Presentations

PP-01: **Neuronal GLT-1 is important for mitochondrial function**

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Background & aim: The glutamate transporter GLT-1 is expressed in neurons, primarily in axon terminals, and in astrocytes. GLT-1 expressed in astrocytes mediates clearance of the majority of released neurotransmitter glutamate, whereas the function of neuronal GLT-1 remains elusive. Since glutamate can also be utilized as an oxidative substrate for the production of energy in mitochondria, we set out to investigate the function of neuronal GLT-1 on mitochondrial energetics in the cerebral cortex, in a conditional neuronal GLT-1 knockout mouse using synapsin 1-Cre (synGLT-1 KO).

Methods: Isolated mitochondria from the cerebral cortex of control and synGLT-1 KO mice were provided pyruvate or glutamate as main respiratory substrates, both in the presence of malate. The rate of oxygen consumption was assessed using a Seahorse XFe96 analyzer and the rate of ATP production was determined by an online luciferin-luciferase assay. Furthermore, the mitochondrial distribution between astrocytes and axon terminals was assessed in the cerebral cortex of control and synGLT-1 KO mice using electron microscopy.

Results: We found that the oxygen consumption rates of isolated mitochondria from control and synGLT-1 KO mice were unaltered when provided pyruvate or glutamate as main respiratory substrates. This was the case for both coupled respiration (stimulated by ADP) and uncoupled respiration (induced by the uncoupling agent FCCP). Interestingly, it was observed that the rate of ATP synthesis was elevated despite the unaffected oxygen consumption. Electron microscopy revealed that the density of mitochondria within axon terminals forming excitatory synapses and perisynaptic astrocytes of synGLT-1 KO mice was increased.

Conclusions: Taken together, our results show that deletion of GLT-1 in neurons affects mitochondrial function. The elevated synthesis of ATP, in the face of unchanged oxygen consumption, may suggest increased mitochondrial efficiency, perhaps as a compensation of reduced availability of glutamate as an oxidative substrate. Furthermore, the increased density of mitochondria in both astrocytes and axon terminals suggests that neuronal GLT-1 contributes to normal synaptic function and may be important for mitochondrial function within both axon terminals and astrocytic processes.

PP-02: Sugar transporters of the major facilitator superfamily: SSM-based electrophysiology reveals common principles and differences

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In contrast to conventional electrophysiology, Solid-supported-membrane (SSM) -beased electrophysiology is designed for the measurements of electrogenic transporters (symporters, exchangers and uniporters) and pumps. Usually these proteins have low turnover rates compared to ion channels. SSM-based electrophysiology compensates for that with a large sensor size which allows for the measurement of up to 10^9 transporters at the same time.

We used SSM-based electrophysiology to characterize proton-coupled sugar transporters of the major facilitator superfamily (MFS). Because a number of structures of MFS sugar symporters have been solved recently, molecular insight into the transport mechanism is possible from detailed functional analysis. We present here a comparative electrophysiological study of the lactose permease ($LacY_{Ec}$), the fucose permease ($FucP_{Ec}$), the xylose permease ($XylE_{Ec}$) and the glucose permease ($GlcP_{Se}$), which reveals common mechanistic principles and differences.

A comparison of the pH dependence of symport at symmetrical pH exhibits broad bell-shaped pH profiles and a decrease at extremely alkaline and at acidic to neutral pH. Experimental evidence suggests that the alkaline pK_{app} is due to H+ depletion at the protonation site, while the acidic pK_{app} is due to inhibition of deprotonation. Since previous studies suggest that a single carboxyl group in LacY (Glu325) may be the only side chain directly involved in H+ translocation and a carboxyl side chain with similar properties has been identified in FucP (Asp46) and XylE (Asp27), the results imply that the pK of this residue is switched during H+/sugar symport in all three symporters.

$GlcP_{Se}$ and $XylE_{Ec}$ are homologs of the human GLUT sugar transporters. Both are equipped with a conserved proton-binding site arguing for an electrogenic transport mode. However, the electrophysiological analysis of $GlcP_{Se}$ reveals important differences between the two GLUT homologs. $GlcP_{Se}$, unlike $XylE_{Ec}$, does not perform steady-state electrogenic transport at symmetrical pH conditions. Furthermore, when a pH gradient is applied, partially uncoupled transport modes can be generated. In contrast to other bacterial sugar transporters analyzed so far, in $GlcP_{Se}$ sugar binding, translocation and release are also accomplished by the deprotonated transporter. Based on these experimental results, we conclude that coupling of sugar and H+ transport is incomplete in $GlcP_{Se}$. This phenomenon can be described by a general 8-state kinetic model.

Results are partly published in the following two papers:

pH Regulation of Electrogenic Sugar/H+ Symport in MFS Sugar Permeases.
10.1371/journal.pone.0156392

A Loose Relationship: Incomplete H^+/Sugar Coupling in the MFS Sugar Transporter GlcP.
10.1016/j.bpj.2017.09.038.

PP-03: **Is SUR1/*ABCC8*, the K_{ATP} channel regulatory subunit, a cryptic peptide transporter married to an ion channel? A speculative hypothesis.**

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K_{ATP} channels, $(SUR1/Kir6.2)_4$, are metabolite sensors that couple the membrane potential of neuroendocrine cells to their metabolic state. Kir6.2/*KCNJ11* is the pore subunit, while SUR1/*ABCC8* is typically considered the regulatory subunit, not a transporter. ATP-binding to the Kir6.2 pore inhibits channel openings, while ATP-binding to the NBD's of SUR1 opposes this inhibition. Eventual understanding of the allosteric protein network(s) that links conformational changes in SUR1 to channel openings requires relating the wealth of structural information on ABC proteins to channel activity. Based on pharmacologic studies of the allosteric interactions between channel agonists (diazoxide), antagonists (glibenclamide; GBM) and ATP we proposed that ATP-bound, more outward-facing conformations of SUR1 bias K_{ATP} channels toward open states [1]. This assertion is supported by recent cryoEM structures of GBM-stabilized, closed K_{ATP} channels with SUR1 in inward-facing states with highest affinity for GBM. A refined structure [2] places the SUR1 GBM-binding site near the middle of the lipid bilayer close to residues in TMD1 and helices 15 and 16 from TMD2 which crossover to form a loop which interacts with NBD1. This positioning is consistent with early mutagenesis data and work aimed at defining the GBM-binding site using an affinity label, ^{125}I-azido GBM, that labeled both SUR1 and Kir6.2. Deletion of 32 residues from the Kir N-terminus, KNtp, to make $\Delta 32$KNtp channels, eliminated Kir6.2 labeling, increased channel open probability and reduced sensitivity to ATP and sulfonylureas. Application of a synthetic 32 residue KNtp peptide to $\Delta 32$KNtp channels partially restored channel activity and ATP and drug sensitivity. Adding peptide to full channels mimicked the deletion of KNtp implying competition between the added peptide and endogenous KNtp for a binding site on SUR1 [3]. In all current K_{ATP} cryoEM structures KNtp is not well resolved, but the irresistible temptation to speculate is supported by unmapped electron densities, potentially KNtp [4], within the SUR1 'transport' cavity. Assuming ^{125}I-azido GBM is relatively immobile in the GBM-binding site, labeling of KNtp implies it must access the cavity defined by TMDs 1 and 2 to approach and be labeled by ^{125}I-azido GBM. In other words, the KNtp binding site on SUR1 is in the transport cavity and SUR1 is a cryptic transporter attempting to move KNtp. In this speculative hypothesis that marries ABC transport functionality directly to channel gating, the ATP-mediated power stroke of SUR1 couples to opening of the pore by tugging on the Kir N-terminus. Supported by NIH DK098647.

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PP-04: **Ligand-induced capacitance changes recorded in HEK293 cells heterologously expressing serotonin transporters**

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The serotonin transporter (SERT) is a secondary active transporter, which clears the extracellular space from its cognate substrate (5-HT) by utilizing the energy stored in the Na+ gradient. Thus, SERT plays a crucial role in curtailing the signals of serotonergic neurons. The partial reactions of the transport cycle of SERT include conformational rearrangements and binding/unbinding reactions of (co-)substrates.

Here we report SERT-specific capacitance changes induced by binding of 5-HT: (i) an initial decrease in apparent capacitance that is induced by adsorption of the charged ligand to its binding site in SERT; followed by (ii) a mono-exponential increase in the capacitance of unknown origin. To elucidate the nature of the latter, we performed capacitance measurements during rapid serotonin application, while substituting the co-substrates with inert ions, which in turn allowed us assigning this capacitance change to the underlying partial reactions in the transport cycle.

We show that capacitance measurements are a powerful tool to study SERT's transport cycle. Here, we provide novel insights into partial reactions, which are inaccessible to other approaches.

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PP-05: **The role of iron in development and activity of osteoclasts**

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INTRODUCTION: Iron is an essential and most abundant trace element in the body. Among the different proteins involved in iron intracellular transport and storage, membrane iron transporters are important to maintain systemic/ cellular iron homeostasis and to prevent metabolic disorders such as anemia and hemochromatosis. Recently, we demonstrated transcriptional regulation of Divalent Metal Transporter (DMT1) and Ferroportin (FPN) during osteoclastogenesis *in vitro*. DMT1 is a membrane transporter for ferrous iron, expressed either on the plasma membrane (in enterocytes) or on endosomal membranes. Most dietary iron is available in the ferric form, as is the absorbed iron, which is transported in the blood stream bound to Transferrin. For transmembranous transport by DMT1, ferric iron has to be reduced by a ferric reductase. FPN is the only known membranous iron exporter so far. To further our understanding of the role of DMT1 and iron in osteoclast (OC) development and activity, we generated osteoclast-specific DMT1-ko mice using the Cre/lox system and developed an *in vitro* iron uptake assay.

METHODS: Bone mass and structure of DMT1(OC)$^{fl/fl}$Cre+ lumbar vertebrae (L3-L5) were evaluated by microCT. OPC from DMT1(OC)$^{fl/fl}$Cre+ were cultured with MCSF and RANKL to assess the effects of DMT1 deficiency on OC proliferation and development. Levels of DMT1 transcripts were determined in OC by RT-PCR. OPCs derived from tdTomato(OC)$^{fl/fl}$Cre+ mice were grown either with or w/o RANKL. At day 5, the cultures were fixed in 4% PFA and cell-specific expression of Cre/Tomato was assessed by confocal microscopy. Osteoclast progenitor cells (OPC) from C57BL/6J mice were cultured with MCSF, RANKL ± iron chelator (Deferoxamine (DFO)). After day 5, OC were incubated for up to 24 h with 55Fe-Holo-Transferrin.

RESULTS: Trabecular thickness in L5 vertebral bodies in male DMT1(OC)$^{fl/fl}$Cre+ mice was significantly decreased when compared to Cre- controls ($P \leq 0.05$). Levels of transcripts encoding DMT1 were decreased by 60% and 30% in OC derived from male and female DMT1(OC)$^{fl/fl}$Cre+ mice, respectively. Development of osteoclasts, as assessed by XTT and TRAP, was not affected by the DMT1 loss. The tdTomato protein was strongly expressed in both macrophage and osteoclast cultures of tdTomato(OC)$^{fl/fl}$Cre+, but not in cells from Cre- control animals. Levels of transcripts encoding TfR1 were significantly increased in osteoclasts cultured in conditions of iron deprivation for 48h ($P < 0.0001$). Iron uptake in OC/DFO was significantly increased compared to OC w/o DFO ($P < 0.0001$).
CONCLUSION: Osteoclast-specific DMT1-ko efficiency is sex-dependent. Trabecular thickness in vertebral spongiosa of male DMT1(OC)$^{fl/fl}$Cre+ mice is decreased in comparison to controls. No changes, however, were detected *in vitro* for cell viability and osteoclastogenesis. Iron deficiency *in vitro* induces a significant upregulation of TfR1 transcripts in OC and consequent increase in Tf-dependent iron uptake.

PP-06: **Novel Cyclin-Dependent Kinase Inhibitors Ribociclib And Palbociclib Show Potential For Pharmacokinetic Drug-Drug Interactions And Overcoming Abcb1- And Abcg2-Mediated Multidrug Resistance**

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Ribociclib and palbociclib are novel cyclin-dependent kinase (CDK) 4 and 6 selective inhibitors that recently gained breakthrough therapy status for advanced breast cancer treatment. With respect to rapid progress of palbociclib and ribociclib in the clinical field, we aimed to identify interactions of these drugs with ABC transporters.

Using established *in vitro* method we show accelerated, ABCB1 inhibitor LY335979-sensitive, basolateral-to-apical transport of ribociclib across MDCKII-ABCB1 cell monolayer, revealing thereby ribociclib, similar to palbociclib, as ABCB1 substrate. The antiproliferative studies supported this finding by demonstrating significantly higher EC_{50} value in ABCB1-, but not ABCG2- or ABCC1-expressing MDCKII cells, than in parental MDCKII cell line. Furthermore, we revealed significant inhibitory effect of ribociclib and palbociclib on ABCB1 and ABCG2 transporters. Inhibition of both transporters by palbociclib and ribociclib was further shown to reverse daunorubicin and mitoxantrone resistance in MDCKII cell lines and human breast carcinoma cell line MCF-7 showing synergistic antiproliferative effect, without affecting the expression of *ABCB1* or *ABCG2*. Additionally, our pilot studies on *ex vivo* isolated mononuclear cells from bone marrow of *de novo* diagnosed acute myeloid leukemia patients confirm these observations and potential of both novel CDK inhibitors to synergize with conventional anticancer drugs.

To sum up, our data indicate an impact of ABCB1 on ribociclib transport across the membranes and high potential of palbociclib and ribociclib for drug-drug interactions (DDI) on ABCB1 and ABCG2 transporters. We further suggest a beneficial MDR reversing potential of both compounds, which might be further exploited in novel anticancer treatment strategies.

This study was supported by the Czech Science Foundation (Grant No. 16-26849S)

PP-07: **Structural and functional interfaces of SLC26 transporters**

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Solute carrier family 26 (SLC26) transporters are widely distributed through all species. Most SLC26 proteins are symporters or exchangers, maintaining anion equilibria across the membranes. The only exception is Prestin (SLC26A5), which acts as a motor protein and plays a key role in the amplification of sound in the cochlea. Mutations in mammalian SLC26 homologs lead to several diseases such as chloride diarrhea (SLC26A3) and deafness (SLC26A4).

To understand the molecular architecture and function of SLC26 proteins, we determined the crystal structure of SLC26Dg from *Deinococcus geothermalis*. The structure of SLC26Dg revealed a membrane domain holding a 7 transmembrane inverted repeat fold which SLC26 proteins share with the dimeric SLC4 and SLC23 families. While SLC26Dg was crystallized as a monomer, crosslinking studies in proteoliposomes indicated a dimeric arrangement in the lipid membrane. Functional interactions in eukaryotic SLC26 dimers have been demonstrated previously, but how the protomers in the SLC26 dimer structurally and functionally interact has remained unclear. In this study, we determined the dimeric, lipid - embedded structure of SLC26Dg using Electron Paramagnetic Resonance spectroscopy. The model suggests a different protomer-protomer interface compared to the interface of the SLC4 and SLC23 family. Furthermore, we provide the first insights into the functional relevance of the dimeric state of SLC26 transporters.

PP-08: **Structural determinants of transport of the Large Neutral Amino Acid Transporter 1 (LAT-1)**

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LAT-1 (SLC7A5) is a sodium-independent exchanger mainly located in the blood brain barrier (BBB) where it mediates the transport of large neutral amino acids, thyroid hormones and prescription drugs. LAT-1 is also upregulated in a variety of cancer types, to provide amino acids that are used as nutrients and signaling molecules for growth. Thus, LAT-1 is an emerging drug target for substrates that can serve as prodrugs with optimal BBB or tumor permeability, or inhibitors that starve cancer cells. Recently, we conducted SAR studies of substrates homologues of LAT-1 that helped understanding the transport specificities of this transporter. Particularly, we have combined computational methods such as homology modeling and virtual screening with experimental testing to identify novel chemical tools for this transporter.

Here, we build on our previous results and construct multiple structural models of LAT-1 representing different conformational states that occur during transport and inhibition and propose a transport mechanism of substrates that provide guidelines to design new substrates with optimized transport properties and inhibitors with higher affinities

PP-09: A novel exosomal cargo protein: SLC22A5, the plasma membrane carnitine transporter (OCTN2).

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Exosomes were discovered long time ago, however initially they were considered nothing more than platelet “dust” or cellular debris. In recent years a growing number of scientific reports describe exosomes as important players in cell-to-cell communication. Indeed, exosomes can move cargos from a donor cell to a target cell with important impact on several physiological and pathological processes such as immune response, angiogenesis, inflammation, cell death, cancer progression and malignancy. Large-scale proteomic studies have been focused on cargo characterization of exosomes derived from body fluids as well as from medium of cell cultures. Among the peptides identified in urinary exosomes by mass spectrometry analysis, some belong to SLC22A5 also known as OCTN2. It is a sodium dependent carnitine transporter which perform the accumulation of carnitine into cells. This process is critical for maintaining the homeostasis of carnitine which is essential for mitochondrial fatty acid oxidation. OCTN2 is ubiquitous but shows a higher expression in kidney, where it realizes the reabsorption of carnitine. In normal condition more than 50% of the required carnitine is furnished by diet. Therefore, in case of non-balanced nutrition, renal reabsorption of carnitine increases trough the augmented expression of OCTN2. It also known that OCTN2 is associated with inflammatory related pathologies such as Crohn's disease. This well correlate with the fact that pro-inflammatory cytokines are able to increase the expression of OCTN2. We have functionally characterized OCTN2 in exosomes derived from human urine and HEK293 cell’s medium. To ascertain if the exosomal OCTN2 is functional or not, the transport properties of the protein has been studied in proteoliposomes reconstituted with the OCTN2 extracted from exosomes. The transport function has been measured as uptake of ^{3}H-carnitine. Transport assays shows that OCTN2 is fully functional, i.e., transport is sodium-dependent and is specific for carnitine. Cell treatment with the pro-inflammatory cytokine, INFγ, increases the level of OCTN2 both in cells and exosomes. Data suggests that exosomal OCTN2 maintain its native functional properties and, hence, its exocellular location may be related to inflammatory states. The fact that OCTN2 is present in urinary exosomes might represent the starting point to discover a novel, non-invasive candidate biomarker for inflammation related pathologies. In this regard we are carrying out a preliminary study on the variability of the expression of OCTN2 in urinary exosomes in the healthy population.

PP-10: **Using conftors and other metrics to describe protein conformation in a standardized way**

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ABC (ATP Binding Cassette) proteins play crucial roles in all type of organisms. Most of them are active transporters and move substrates, including nutrients, hormones, and xenobiotics, across the membranes. The altered function and expression of ABC proteins in humans are connected to various pathological phenomena. Due to the revolution in cryo-electron microscopy, there is an increasing number of experimental ABC protein structures available. This raised the necessity of the identification of differences between conformations and assessing their validity. In addition, to understand the similarities and differences in their mechanism of action, the characterization and comparison of specific features of ABC structures are required. Since this characterization can be improved by standardized measures, we defined special vectors (conftors) which describe the relative orientation of transmembrane and nucleotide binding domains of ABC proteins. These vectors are sensitive to small differences in conformations, therefore can highlight deviations in a structure compared to a set of conformations. We also assessed the membrane insertion of the available ABC conformations by bioinformatics tools and molecular dynamics simulations, as well as solvation energy and electrostatics calculations. Our results provide measures that can be used to structure validation and identification of crucial differences between conformations and molecular dynamics trajectory. Therefore these metrics allow us to better understand the structural features of ABC exporters. In addition, the concept of standardized measures can be applied to other type of proteins as well.

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PP-11: **The cysteine-glutamate exchanger (xCT, slc7a11) is expressed in significant concentrations in a subpopulation of astrocytes in the mouse brain**

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The cystine-glutamate exchanger (xCT) promotes glutathione synthesis by catalyzing cystine uptake and glutamate release. The released glutamate may modulate normal neural signaling and contribute to excitotoxicity in pathological situations. Uncertainty, however, remains as neither the expression levels nor the distribution of xCT have been unambiguously determined. In fact, xCT has been reported in astrocytes, neurons, oligodendrocytes and microglia, but most of the information derives from cell cultures. Here, we show by immunohistochemistry and by Western blotting that xCT is widely expressed in the central nervous system of both sexes. The labeling specificity was validated using tissue from xCT knockout mice as controls. Astrocytes were selectively labeled, but showed greatly varying labeling intensities. This astroglial heterogeneity resulted in an astrocyte domain-like labeling pattern. Strong xCT labeling was also found in the leptomeninges, along some blood vessels, in selected circumventricular organs and in a subpopulation of tanycytes residing the lateral walls of the ventral third ventricle. Neurons, oligodendrocytes and resting microglia, as well as reactive microglia induced by glutamine synthetase deficiency, were unlabeled. The concentration of xCT protein in hippocampus was compared with that of the EAAT3 glutamate transporter by immunoblotting using a chimeric xCT-EAAT3 protein to normalize xCT and EAAT3 labeling intensities. The immunoblots suggested an xCT/EAAT3 ratio close to one (0.75 ± 0.07; average ± *SEM*; $n = 4$) in adult C57BL6 mice. Conclusions: xCT is present in select blood/brain/CSF interface areas and in an astrocyte subpopulation, in sufficient quantities to support the notion that system x_c^- provides physiologically relevant transport activity.

PP-12: **Engineering the transport of dicarboxylic acids in yeast *Saccharomyces cerevisiae***

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Secretion of products during fermentation presents multiple advantages in comparison to intracellular accumulation, such as reduced toxicity, increased titers, and simpler purification process. We characterized six carboxylic acid transporters from different organisms using *Xenopus* oocytes (frog eggs) expression system. All the tested transporters, included putative candidates that we identified through genomic co-localization and homology searches, were capable of exporting dicarboxylic acids, albeit at different rates. The previously reported transporter from *Schizosaccharomyces pombe* SpMAE1 was the most efficient exporter of malate, fumarate, and succinate. Next, we overexpressed each of the dicarboxylic transporters in yeast *Saccharomyces cerevisiae* designed to overproduce malate by overexpression of *MDH3* and *PYC1/PYC2* genes and deletion of *PDC* genes. Again, SpMAE1 was the most efficient transporter, increasing malate titer 8-fold, and most interestingly, it did not result in a growth defect as the other transporters compromised the growth. We discovered that SpMAE1 had a different mechanism than the other transporters and using this knowledge, we engineered it to improve the flux even further.

PP-13: **Single-molecule microscopy to study oligomerization of membrane transporters in live cells**

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How proteins interact is a fundamental question in cell biology, and protein oligomerization from the perspective of protein evolution and new opportunities for functional control has been an intense area of research. Neurotransmitter transporters are transmembrane proteins present in the plasma membrane of presynaptic nerve terminals and aid in the removal of specific neurotransmitters from the extracellular space of the synapse, thereby terminating their actions. We are interested in the oligomerization behavior of these transporters and its underlying mechanisms.

We use state of the art single-molecule microscopy techniques including single-molecule brightness analysis, single-particle tracking and super-resolution microscopy to determine the subunit stoichiometry, mobility, interaction kinetics and nanoscopic organization of dopamine transporter (DAT), GABA transporter (GAT) and serotonin transporter (SERT). Our results suggest that the oligomeric distribution of DAT and GAT is different from that of SERT. DAT and GAT show a major dimeric fraction as opposed to multimers (up to pentamers) seen with SERT [1]. Earlier, our group has shown that in case of SERT, oligomerization is equilibrated at the level of the endoplasmic reticulum (ER). Oligomers are kinetically trapped at the plasma membrane indicating the role of subcellular compartments in oligomerization behavior. The observed difference in oligomerization behavior was attributed to the differences in PIP_2 levels and henceforth its interaction with the SERT at the ER and the plasma membrane [2]. Interestingly, we observe that in case of DAT, though the dimers of DAT are stable at the plasma membrane, perturbation of PIP_2 levels seems to have no significant effect. We are now investigating the underlying mechanisms of oligomerization in DAT and GAT.

1. Anderluh, A., et al., *Single molecule analysis reveals coexistence of stable serotonin transporter monomers and oligomers in the live cell plasma membrane.* J Biol Chem, 2014. **289**(7): p. 4387-94.
2. Anderluh, A., et al., *Direct PIP2 binding mediates stable oligomer formation of the serotonin transporter.* Nat Commun, 2017. **8**: p. 14089.

PP-14: An electrophysiological approach to decipher the function of EL4 of the serotonin transporter in the transport cycle by antibody based inhibition

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Solute carrier (SLC) transporters are responsible for the transport of ions, amino acids, and neurotransmitters, which cannot diffuse passively across cellular membranes. Monoamine transporters, a subfamily of the solute carrier 6 (SLC6) family, are located in the plasma membranes of monoaminergic neurons to terminate monoaminergic neurotransmission and replenish monoamine neurotransmitter supplies. The serotonin transporter (SERT) is a monoamine transporter, reuptakes of serotonin (5-HT) from the synapses. SERT is an integral membrane protein consist of 12 transmembrane segments that are linked by 6 extracellular (EL) and 5 intracellular loops (IL). Biochemical studies have shown that EL4 is taking part in the conformational changes visited during 5-HT transport, together with transmembrane domains 7 and 8 (TM7 and TM8). Studies on the functional role of these structural motifs mostly depend on site directed mutagenesis. However, employing this method often result in a loss of function as a result of impaired protein folding and plasma trafficking. Hence, a tool is required that allows to investigate the functional role of a structural motif of interest while protein folding and trafficking remain unaffected. 5-HT induced currents were recorded by whole-cell patch-clamp technique in human SERT (hSERT) expressing HEK293 cells upon the application of antiSERT-EL4 antibody. In addition, membrane capacitance measurements were performed to assess ligand-binding (5-HT and anti-SERT EL4) to SERT. Inhibition of EL4 of SERT by antiSERT-EL4 antibody reduces the membrane capacitance and blocks the 5-HT transport in a dose-dependent manner. The association and dissociation rates of the antibody (Kon and Koff) are calculated as 3,055 ± 0,631 M-1*s-1x 107 and 1,352 ± 0,082 s-1 respectively. Our data indicate that employing antibodies as tools in electrophysiology is a promising approach to study structural motifs of transporters. Blockage of the 5-HT transport upon the restriction of EL4 movement shows that EL4 has a role in the conformational changes on SERT, required for 5-HT transport. Further experiments are planned by producing Fabs from antiSERT-EL4 antibody to eliminate the possibility of antibody dimerization.

PP-15: A genome-wide FACS based screen in macrophages uncovers genes involved in phagocytosis and cellular pH homeostasis.

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Macrophages embody the first defense mechanisms of the immune system during the invasion of pathogens. In our recent publication, Sedlyarov et al. performed a FACS-based solute-carrier (SLC)-focused CRISPR/Cas9 mediated mutagenesis screen that employs pH sensitive reporter latex beads taken up by macrophages. Interestingly, we found that the bicarbonate transporter SLC4A7 is not only critical for phagosome acidification, a key step in the deactivation of pathogens, but also that it acts through homeostatic regulation of the cytoplasmic pH. This discovery highlights the fact that pH is one of the key drivers of cellular homeostasis. Thus, it is involved in the regulation of many cellular processes, which makes it an interesting target for future studies and potential new therapies.

We now follow up by expanding our approach to a genome-wide scale, targeting every human gene by four guide RNAs and thereby apply FACS-based CRISPR/Cas9 mediated mutagenesis screening in different human monocytic cell lines. Our dual labeled pH sensitive latex beads enter macrophages during phagocytosis and serves as a reporter to discriminate cells at different stages of the phagocytic process. FACS sorting of defined populations and subsequent NGS analysis allows us to map the genes responsible for different stages of phagocytosis and for phagosome acidification. Furthermore, employing new pH sensitive reporters, co-culturing different cell types and specific changes of the cultural milieu will allow us to look even deeper into cellular processes, find the genes and SLCs involved in their regulation and employ them for future targeted therapies.

PP-16: **PKC-stimulated dopamine transporter internalization: Interdependent roles of Rin, Ack1 and transporter amino- and carboxy termini**

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Dopamine (DA) signaling is critical for movement, motivation, and reward. Synaptic DA availability is spatially and temporally limited by presynaptic DA clearance, mediated by the DA transporter (DAT). DAT coding variants in patients with DA-related neuropsychiatric disorders clearly illustrate that DAT is vital for DA homeostasis and DA-related behaviors. DAT plasma membrane expression is highly dynamic; DAT constitutively recycles to and from the plasma membrane, and protein kinase C (PKC) activation significantly stimulates DAT internalization and diminishes both DAT surface levels and function. We independently reported that both the neuronal GTPase, Rin (RIT2), and the non-receptor tyrosine kinase, Ack1 (TNK2), play requisite mechanistic roles for PKC-stimulated DAT internalization.

Importantly, although PKC-stimulated DAT internalization requires Rin and Ack1, the closely related serotonin transporter (SERT) neither interacts with Rin, nor is sensitive to Ack1 inactivation. Despite their clear role in regulated DAT endocytosis, it is currently unknown whether Rin and Ack1 are mechanistically linked to promote PKC-stimulated DAT internalization. Moreover, although Rin interacts directly with DAT, it is unknown whether DAT intracellular domains act synergistically to facilitate DAT/Rin interactions and regulated DAT internalization. Here, we used GTPase mutants and shRNA-mediated gene knockdown to test whether there is a mechanistic linkage between Rin, Ack1, and DAT, and used a chimeric protein approach to determine the DAT structural determinants required for Rin and Ack to impact DAT internalization. Preliminary results indicate that Rin is required for PKC- dependent Ack1 inactivation, placing Rin upstream of Ack1 in the PKC-stimulated DAT regulatory pathway. Ongoing experiments will examine how Rin knockdown impacts PKC-stimulated Ack1 inactivation and DAT internalization. DAT/SERT chimera studies, in which DAT N-, C- or both termini were replaced with cognate SERT domains, revealed that the DAT N-terminus, but not the C-terminus, is required for PKC-mediated internalization, but not for internalization in response to Ack1 inactivation. In contrast, both DAT N- and C-termini are required for both PKC- and Ack-dependent internalization. Pulldown studies using an extracellular bungarotoxin binding site in DAT demonstrate that PKC activation decreases the DAT/Rin association, and that PKC-dependent DAT/Rin dissociation requires the DAT N- terminus. Future FRET experiments will test whether the DAT termini are required for the DAT/Rin direct interaction. Taken together, these studies will further elucidate the interplay between two known signaling pathways that control DAT surface expression, and test whether the intracellular termini synergistically coordinate for DAT internalization in response to these signaling events.

PP-17: **Characterization of the CFTR chloride channel**

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Cystic fibrosis (CF) is a severe, monogenic disease caused by the mutations of the cystic fibrosis conductance regulator (ABCC7/CFTR), a member of the ATP Binding Cassette (ABC) protein superfamily. Decrease in the functional expression of the CFTR chloride channel leads to reduced chloride conductance in the epithelia resulting in the disturbance of the salt and water homeostasis. There are small drug molecules to correct the protein folding and restore the function, but their therapeutic effect is small. For the development of more efficient drug molecules, a high resolution protein structure is required. Our goal is to characterize the CFTR structure and dynamics based on both experimental and homology models, using *in silico* methods. Even though the recently published zebrafish cryo-EM CFTR structure (PDBID:5W81) is solved in the active (ATP-bound, phosphorylated) conformation, it lacks an open channel for chloride permeation. The stable closure may arise from the disturbed transmembrane helix 8 and the loosely associated NBDs, which may result in the lack of required conformational changes to form an open channel. To examine the dynamics of the channel opening of the ATP-bound CFTR structure, we performed molecular dynamics (MD) simulations and analyzed the generated conformations to describe the properties of the possible pathways through the protein. We characterized the tunnels in the conformation generated by MD. Our results suggest that the structure is either rare under physiological conditions or it could have been distorted due to the conditions required for cryo-EM structure determination. To overcome the lack of channel opening we generated a CFTR model based on MRP1 (PDBID: 6BHU), with continuous transmembrane helix 8. The simulations of the remodeled CFTR exhibit feasible channel openings both at the extracellular and at the intracellular sides in contrast to the simulations with the original cryo-EM CFTR structure. A detailed analysis of the tunnel-lining residues and modeling the passage of the chloride ion are in progress.
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PP-18: MCT2-mediated MOG transport dictates intracellular target engagement to drive toxicity in cancer cells

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α-Ketoglutarate (αKG) is a central metabolic node that plays important physiological roles in processes ranging from TCA and amino acid metabolism to epigenetics. The αKG analogue NOG (*N*-oxalylglycine) has been widely used to study αKG-dependent dioxygenases such as hydroxylases and demethylases, and its prodrug, DMOG (dimethyloxalylglycine), is a common tool in the study of HIF1α signalling. Here we demonstrate that, in buffered aqueous solutions, DMOG is rapidly hydrolysed to form the monocarboxylate methyloxaylglycine (MOG). MOG shows toxicity across a wide range of cancer types, however this toxicity is selective. Using gene expression analysis, we show that this selectivity is based on expression of the monocarboxylate transporter, MCT2, which transports MOG into cells where it is rapidly de-esterified to form NOG. In the absence of MCT2, low intracellular concentrations of NOG can interact with high affinity targets such as PHDs, allowing it to stabilise HIF in all cell lines. However, in cells expressing MCT2, NOG is able to accumulate to a level at which it can engage multiple low affinity targets. We use a range of metabolomics techniques to show that by inhibiting these targets, NOG has major effects on alpha-ketoglutarate metabolism, leading to significant impairments in TCA cycle flux with subsequent decreases in respiration and ATP production, which ultimately drive the observed toxicity. These findings demonstrate that MCT2 expression levels define intracellular concentrations of NOG, and thereby dictate the range of targets with which the compound is able to engage, illustrating the importance of understanding transporter-mediated drug-uptake and the impact it can have on compound specificity *in vivo.*

PP-19: **Absence of BSEP (ABCB11) protects MDR2 (ABCB4) KO mice from cholestatic liver and bile duct injury through anti-inflammatory bile acid composition and signaling**

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Background: Bsep (Abcb11) KO mice are protected from acquired cholestasis by metabolic preconditioning resulting in a hydrophilic bile acid (BA) pool with formation of tetrahydroxylated bile acids (THBAs). We aimed to explore whether increased BA detoxification/THBAs alters inflammatory signaling, thereby improving liver injury in the Mdr2 (Abcb4) KO mouse model of sclerosing cholangitis.

Methods: Cholestatic liver injury, hepatic inflammation and fibrosis in Mdr2/Bsep DKO and Mdr2 KO mice was studied for comparison. In addition BDL WT and Mdr2 KO mice were treated with a THBA. Gene expression profiles of inflammatory/fibrotic markers were investigated by RT-PCR and Western blotting. RORgt+ and FOXP3+ T cells from liver were quantified by FACS. In vitro, the impact of THBA on EGR1 signaling in IHH cells and RORgt as well as NFkB signaling in Jurcat cells (stably transfected with GFP -NFkB) were analyzed.

Results: In contrast to Mdr2 KO, DKO mice displayed increased BA hydroxylation and lacked histological features of sclerosing cholangitis. 67% of serum BAs in DKO mice were polyhydroxylated, with THBAs being most prominent, while Mdr2 KO mice had no such BAs. In contrast to profoundly increased gene expression of inflammatory and fibrotic markers (F4/80, Tnf�� Mcp1, Desmin, Col1a1; $p<0,05$) in Mdr2 KO, no increases were seen in DKO. EGR1 (a key regulator of hepatocellular cytokine secretion) protein levels were profoundly reduced in livers of DKO compared to Mdr2 KO mice. In IHH cells, THBA reduced mRNA of Egr1 and its pro-inflammatory downstream targets (IL8, Icam, Cxcl2; $p<0,05$). Increased levels of PHBAs were associated with reduced RORgt+ (regulator of TH17 cell differentiation) cells but increased FOXP3+ (Treg differentiation) within the CD4+CD3+ T cell population (50% RORgt+ and 5% FOXP3+ in Mdr2 KO versus 10% RORgt+ and 30% FOXP3+ cells in DKO). In vitro, THBA attenuated RORgt signaling at mRNA (IL23 -55%, TGF�-25%; TH17 related cytokines) in Jurcat cells. It also attenuated CDCA-induced NFkB activation in GFP-NFkB transfected Jurcat cells. THBA feeding reduced inflammatory (IL1b, Cxcl1 by 50%) and fibrotic (Col1a2 by 80%) genes in Mdr2 KO. In line, in WT BDL mice, bile infarct size, F4/80 immunostaining and inflammatory gene expression (F4/80 -50%, Cxcl1 -55% and Cxcl2 -75%; $p<0,05$) were also profoundly reduced by THBA.

Conclusion: Increased formation of THBA (due to absence of Bsep) or THBA administration represses key pro-inflammatory signals such as EGR1, NFkB and RORgt in hepatocytes and immune cells. These changes protect Mdr2 KO mice from cholestasis-associated inflammation and fibrosis. Therefore, THBA and their downstream targets may be a new potential treatment strategy for cholestatic liver diseases.

PP-20: **Age- and sex-specific perturbations in dopamine transporter function induced by food restriction and exercise**

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Eating disorders, which are 2.5-fold more prevalent in females than males, typically emerge during adolescence. Afflicting at least 3 percent of teenagers, eating disorders such as anorexia nervosa, bulimia nervosa, and binge eating disorder entail severe health consequences in addition to their psychological toll. Yet no effective treatments for eating disorders exist. An established regulator of both eating behaviors and physical activity, the dopaminergic system undergoes a sensitive maturation period during adolescence. However, studies into the role of the dopaminergic system in ontogeny of eating disorders are lacking, as are investigations into dopaminergic system maturation in adolescent females. Here we measured function of the dopamine transporter (DAT), a critical regulator of dopaminergic signaling, using both in vivo high-speed chronoamperometry and locomotor assays of acute cocaine response. Employing an activity-based anorexia paradigm for 4-5 days, we investigated how food restriction, free exercise on a running wheel, or the combination thereof impacted DAT function in adult (postnatal day 90) and adolescent (postnatal day 30) Sprague-Dawley rats of both sexes. Food restriction alone or with exercise in adolescent rats of both sexes produced leftward shifts in the dose-response to the locomotor-promoting effects of cocaine. In adults, females exhibited an expected increase in cocaine-induced locomotor response relative to males, but no clear effects of food restriction and/or exercise were observed. Clearance of dopamine in dorsal striatum was not significantly altered by food restriction and/or exercise in adults. In adolescents, food restriction alone slowed dopamine clearance, but when combined with exercise, dopamine clearance was further attenuated in males yet returned to control rates in females. Together, these findings suggest that the enhanced dopamine clearance during adolescence, and accompanying impairments from food restriction, may help explain the common onset of drug abuse and eating disorders during this vulnerable age. Therefore, drugs that enhance dopamine uptake or otherwise reduce dopamine signaling duration may prove efficacious in the treatment of emerging adolescent eating disorders. Ongoing experiments are evaluating striatal DAT expression in these animals, and future experiments will focus on optimizing longer term food restriction and exercise conditions in adolescents to facilitate investigation of pharmacologic interventions.

PP-21: **A functional survey of Solute Carrier-drug interactions**

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Despite the increasing evidence that several drugs depend on carried-mediated transport as the predominant entry method into the cell, the question of whether all drugs require a transporter to cross membranes remains a matter of debate. To gain insight into this issue and identify novel transporter-drug associations, we performed a large series of genetic screens using a set of 60 cytotoxic drugs, covering a broad chemical space, in the haploid human cell line HAP1. A SLC-focused CRISPR/Cas9 lentiviral library, targeting 388 human SLC genes with multiple guides per gene, was generated and allowed us to identify transporters whose absence induced resistance to the drug tested. Both known (e.g. anti-folates and the folate transporter SLC19A1, nucleoside analogs and SLC29A1) as well as novel interactions were identified for a significant proportion of the compounds screened, suggesting an important and widespread role for SLCs in determining cellular activity and uptake of cytotoxic drugs and providing a large set of SLC-drug associations for a diverse set of clinically relevant compounds.

PP-22: **Dopamine transporter is dysregulated on the peripheral immune cells of drug naïve Parkinson's Disease patients**

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Parkinson's Disease (PD) is characterized by loss of substantia nigra (SN) dopaminergic neurons, resulting in reduced CNS dopamine transmission. PD is widely thought to start in the periphery; however, whether and how PD affects peripheral dopamine transmission remains unknown. Peripheral immune cells express key dopaminergic proteins, including dopamine transporter (DAT) and tyrosine hydroxylase (TH). Therefore, it is possible that the peripheral dopamine system is affected by PD pathology. We hypothesized that peripheral immune cells of PD patients exhibit dysregulated dopamine homeostasis. Using flow cytometry assay we found human peripheral blood mononuclear cells (PBMCs) constitutively express DAT and TH with 90% of CD14+ cells expressing both markers. Upon examining PBMCs of PD patients receiving a variety of treatments, we found that DAT/TH positive PBMCs are elevated in PD patients compared to healthy controls (n=51 independent biological replicates, $P<0.05$) irrespective of the treatment modality applied. Importantly, in drug naïve patients diagnosed with PD we observed the highest increase in DAT/TH positive monocytes (n=5, $P<0.05$) compared to both treated PD patients and healthy individuals, suggesting observed changes correlate with disease pathology and not treatment interventions. Collectively, these data suggest a system-wide dysregulation of the dopamine system on peripheral immune cells in PD. To further understand the functional consequences of increased DAT+/TH+ PBMCs, we asked if DAT function was altered on PD monocyte-derived cells. Live cell fluorescence microscopy and biochemical analysis confirmed healthy human monocyte-derived macrophages (MDM) express membrane-localized, canonically functional DAT (Km=3.2mM). Surprisingly, relative to healthy age-matched controls, PD patients' MDMs exhibited dramatically elevated DAT-mediated substrate uptake and increased membrane localization ($p<0.0001$, n=5 biological replicates). Taken together, these data are consistent with the interpretation that in PD peripheral dopamine homeostasis is dysregulated. Importantly, this presents PBMC DAT/TH as a potential biomarker for PD and suggests the peripheral dopamine system is functionally linked to the CNS dopamine system. Future work will aim to validate this biomarker and investigate the mechanistic connection between peripheral-CNS dopamine systems to elucidate the pathophysiological role of dysregulated monocyte dopamine transmission in PD.

PP-23: **Differences in Drug Transporter Expression in Human Monocytes, Macrophages and THP-1 Cells**

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Background: Drug transporters are well characterized in tissues like liver, intestine or the blood-brain-barrier. In contrast, information on their expression and function in mononuclear blood cells is still limited. However, some reports indicate a highly differentiated expression of some transporters between monocytes and monocyte-derived macrophages. We therefore analyzed the expression of 7 ABC-transporters (ABCB1, ABCC1-5, ABCG2), 24 SLC-transporters (SLC15A1 and 2, SLC16A1, SLC22A1-8, SLC22A11, SLC22A16, SLC22A18, SLC28A1-3, SLC29A1-4, SLC47A1, SLC51A/B) and 10 SLCO-transporters (SLCO1A2, SLCO1B1, SLCO1C1, SLCO2A1, SLCO2B1, SLCO3A1, SLCO4A1, SLCO4C1, SLCO5A1) in monocytes and macrophages and evaluated the monocytic THP-1 cell line as a monocyte/macrophage in vitro model concerning these transporters. Finally, the impact of an LPS-mediated activation on the transporter expression was studied.

Methods: Experiments were carried out using human monocytes isolated from buffy coats and monocyte-derived macrophages differentiated using GM-CSF. In addition, native THP-1 cells as well as THP-1 derived macrophages (differentiated using PMA) were used. For activation monocyte- and THP-1-derived macrophages were treated with 100 ng/ml LPS for 24h. Drug transporter expression was analyzed on mRNA level using custom made Taqman® low density arrays.

Results: All analyzed ABC-transporters are expressed in monocytes and THP-1 cells. In contrast, only 17 of the 24 analyzed SLC-transporters were detected, for example mRNA expression of SLC22A1 (OCT1), SLC28A3 (CNT3), SLC29A3 (ENT3) and SLC47A1 (MATE1) was found. Concerning the SLCO-transporters SLCO1A2, SLCO1B1 and SLCO1C1 were absent in monocytes and THP-1. In addition, transporter expression was studied during differentiation. While ABCC1, 3, 4, 5, ABCG2 and SLCO2B1 were upregulated in both models, ABCB1 and ABCC2 mRNA levels were reduced in monocyte-derived macrophages and upregulated in THP-1-derived macrophages. Finally, the effect of LPS on transporter expression was studied in monocyte and THP-1-derived macrophages revealing an enhanced expression of ABCC1, ABCC3, SLCO3A1, SLCO4A1 and SLC51A/B in both models.

Conclusions: Taken together, we analyzed several drug transporters in monocytes and monocyte-derived macrophages, demonstrating significantly regulated expression of transporters like ABCG2 and SLCO2B1. In addition, we demonstrated that THP-1 and THP-1-derived macrophages are a suitable model to further investigate the impact of these transporters in monocytes and macrophages.

PP-24: **The Extended SLC Atlas**

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Transport of solutes across various biological membranes is essential to maintain cellular homeostasis and metabolism, and its dysfunction plays a pivotal role in the development of various diseases. The Solute Carrier (SLC) nomenclature system aims to enumerate and categorize mammalian secondary active and facilitative transporters. Proteins have been originally added to the SLC superfamily on a case-by-case basis, and grouped into families based on protein sequence identity. However, as of yet, no attempt has been made to systematically collect all SLC-like transporters from the human genome. Some resources, such as the Transporter Classification Database (TCDB) or TransportDB categorize transporters from all kingdoms of life, without any special focus on human health or disease. In order to generate a complete overview of SLC-mediated transport processes in human cells and biologically relevant organisms, and to facilitate integrative approaches in biology, we have collected all known proteins that could qualify as SLC-like transporters from selected species, according to our current knowledge. To this end, an inclusive bioinformatics approach was taken, combining information from the TCDB, Pfam and Uniprot databases, to yield a potentially complete list of SLC-like transporter proteins. The task is considerably challenging given the heterogeneity of SLC superfamily members.

Our meta-analysis started by a manual curation of all TCDB families and corresponding Pfam domains to select those that mention membrane transport activity. ATP-dependent transporters and channels were excluded. In a second step, in addition to using existing Pfam models, hidden Markov models (HMMs) were generated for TCDB families that are not covered by available Pfam models. HMM searches were performed for seven clinically relevant organisms (*H. sapiens, R. norvegicus, M. musculus, G. gallus, D. rerio, D. melanogaster, C. elegans*) on complete proteomes according to the Uniprot database, including TrEMBL sequences. The resulting 4032 sequences were clustered according to their HMM fingerprints. Using this method, we were able to reproduce the existing SLC families as well as to uncover several novel transporter protein families.

Overall, our search revealed ~140 transporter-like proteins outside the current SLC families, which could be grouped into ~50 protein families. A thorough and ongoing literature search provided, until now, evidence for transport function of 22 of these "novel" proteins, of which 14 new SLC families have already been assigned by the Human Gene Nomenclature Committee (HGNC). The new families have been included in the BioParadigms SLC Tables (http://www.bioparadigms.org/) and feature amino acid and sugar transporters, ion exchangers and transporters of hydrophobic/amphipathic molecules, including cholesterol. Our study highlights that the landscape of secondary and facilitative transporters is likely much wider than previously anticipated.

PP-25: **Astrocytic transport processes contribute to neuronal oscillations in vivo**

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Oscillatory changes in the activity of neurons are a fundamental hallmark of the brain. Since these oscillatory activities are not limited to single neurons, instead they extend to large brain areas, their appearance can be detected even from outside the skull. The vast amount of data collected from such measurements since the advent of experimental brain physiology collectively demonstrated that a wide range of crucial physiological functions from memory formation up to behavior can be attributed to neuronal oscillations at different frequency ranges. Despite their unambiguous importance, however, it is still largely unknown what mechanisms are responsible for the emergence and spreading of oscillatory network activity. Here we demonstrate that astrocytes, the increasingly recognized modulators of neuronal activity are largely responsible for the initiation and/or spreading of oscillatory neuronal activity. Using a transgenic rat line expressing a calcium sensitive fluorescent protein in both astrocytes and neurons, we in vivo explored the correlation between astrocytic activity and the emergence of two different essential neuronal oscillations, the low-frequency, physiological slow wave activity and the high-frequency, pathophysiological epileptic seizures. Strikingly, we observed that the astrocyte network display synchronized recurrent activity in vivo, coupled to both neuronal activities. Importantly, the astrocyte network activity does not merely follow neuronal activation. Instead, we present evidence that extensive synchronization of the astrocytic network precedes the spatial build-up of neuronal synchronization. We show that astrocytic transport processes through gap junctions and astrocytic GABA transporters are the key players that contribute to the emergence and maintenance of neuronal synchronization. Confirming this notion, we demonstrate that blockade of astrocytic gap junctional communication reduces or inhibit neuronal activity in both slow-wave activity and epilepsy. These in vivo findings conclusively suggest a causal role of the astrocytic syncytium in the generation of different forms of neuronal oscillations.

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PP-26: **The human serotonin transporter in an outward-occluded state**

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The human serotonin transporter (hSERT) facilitates the reuptake of serotonin from the synaptic cleft after signaling and is therefore an important drug target. Interacting compounds can have different effects on the transport mechanism ranging from inhibition (e.g. antidepressants) to reverse transport (e. g. psychostimulants).

The mechanistic distinction between transporter substrates and inhibitors is not always possible based on the physicochemical properties of these compounds.

For example, it remains to be established whether the closure of the extracellular pathway and the opening of the intracellular pathway facilitated by substrate can also occur when such inhibitors are bound. It therefore seems necessary to pinpoint the molecular features of the substrate required for the transport-related conformational change, and in particular, the outward-open to outward-occluded transition.

A recently reported X-ray structure of the outward-open state of hSERT provides a starting point for molecular dynamics (MD) simulations of this transition. Initial MD simulations revealed stability and convergence of this conformation over time, but even in complex with the endogenous ligand serotonin (poses gained from docking studies), no stable transition events have been established yet. As the endpoint of this pathway closure event is not yet known, we present a homology model of hSERT based on a combination of two templates (hSERT, outward-open and the bacterial leucine transporter LeuT, outward-occluded) in an outward-occluded state. This approach allows to benefit from the structural information enlightened by X-ray crystallography and to generate a new conformational state of hSERT at the same time. Thorough validation including several scoring methods and careful refinement steps lead to a well-chosen set of final models.

An induced-fit docking study with serotonin and a subsequent common scaffold clustering approach reveal the binding pose of the endogenous transporter substrate in the central binding site of this outward-occluded conformation.

The model and the ligand are stable over time in microsecond long free MD simulations which allow detailed insights into the behavior of the gating residues. Ongoing induced-fit docking and MD simulations comparing substrates and inhibitors will lead to a better understanding of the transporter mechanism

PP-27: **Elucidation of self-mediated enhancement of dopamine transport by the dopamine transporter which can be modulated by extracellular gate and N-terminal residues**

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The dopamine (DA) and serotonin (5-HT) transporters (DAT and SERT respectively) are members of the SLC6 family of neurotransmitter: sodium symporters (NSSs). DAT and SERT clear substrate from perisynaptic spaces by the generally accepted alternating access mechanism in which the transporters transition between outward-facing and inward-facing conformational states. This mechanism involves a sequential closing and opening of extracellular (EC) and intracellular (IC) gates along the permeation pathway. The EC and IC gates are almost exclusively composed of acidic and basic residues capable of interacting through salt bridge formation, closing the primary substrate binding site to the intracellular and extracellular milieus.

Previously, we identified that the human SERT acidic/basic residue pair is critical for the recognition of 3,4-methylenedioxymethamphetamine (MDMA) as a substrate. In contrast, mutations in the EC gate had no effect on 5-HT recognition. Our current efforts focus on determining if the EC gate of the human DAT has similar mechanistic characteristics for substrate selectivity. While investigating that possibility, we identified a previously unreported phenomenon of self-mediated enhancement of DA transport in hDAT expressing cells. In homologous competitive uptake experiments utilizing [^{3}H]DA, a 200% to 300% increase in [^{3}H]DA uptake is observed upon addition of micromolar concentrations of unlabeled DA. This enhancement is further modulated by the identity of the acidic EC gate residue. Substituting the Asp with the polar amino acid Asn (D476N) produces a 600% increase in [^{3}H]DA uptake. Experiments utilizing heterologous competition with [^{3}H]DA and the known hDAT substrates 1-methyl-4-phenylpyridinium (MPP^+), 4-(4-dimethylamino)phenyl-1-methylpyridinium (APP^+) and tyramine indicate that the enhancement effect is not exclusive to DA nor is it observed with all DAT substrates.

Notably, substitution of several phosphorylatable N-terminal residues to alanine virtually eliminates the DA self-enhancement phenotype. The apparent ability of DA to augment its own uptake is consistent with reports proposing a second substrate binding site (S2) that can act as a trigger for release of substrate from the primary DA binding site (S1). However, the presence of this S2 site remains controversial. Continued investigation of this phenomenon may provide insight into molecular determinants on both the substrate and transporter that are important for substrate identification and translocation.

PP-28: **Loss of function variants in monoamine transportes as a risk factor for neuropsychiatric disease? – Insights from a population based case-cohort sample**

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It is well established that disturbances in monoaminergic neurotransmission are involved in the pathophysiology of many neuropsychiatric diseases including ADHD, depression, autism spectrum disorder, bipolar disorder and schizophrenia. Some of the most powerful regulators of monoaminergic signaling are the monoamine transporter proteins that includes the intracellular vesicular monoamine transporter 2 (VMAT2) which sequesters monoamines into synaptic vesicles and the plasma membrane transporters of norepinephrine (NET), serotonin (SERT), and dopamine (DAT) that ensures neurotransmitter reuptake which is critical for terminating neurotransmission and for maintaining a synthesis independent supply of neurotransmitter. Despite their important function, little is known about how rare genetic variants of monoamine transporters may contribute to the genetic architecture of neuropsychiatric diseases at a populational level, but constrain metrics indicate a negative selection against loss of function variations and/or missense mutations. We proposed the hypothesis that disruptive mutations in monoamine transporters might increase the risk of neuropsychiatric disease, and that this might at least in part contribute to the negative selection against loss of function variants. To test this, we investigated the occurrence and allelic diversity of loss of function variants in a population based case-cohort sample encompassing five major mental disorders: ADHD, autism, schizophrenia, bipolar disorder, or depression.

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PP-29: **Making Matters Worse: The Synergistic Effects of Serotonin and Histamine in Depression**

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Neuroinflammation is associated with changes to the central nervous system (CNS) and plays a role in the pathology of depression along with many other neurological diseases. By targeting the neurotransmitter systems of serotonin and histamine we aim to understand the neurochemical underpinnings of depression. Serotonin has long been hypothesized to play a role in depression as serotonin signaling is often impaired during depression. Histamine has a well-established role in peripheral inflammation and novel data showing that histamine release inhibits serotonin signaling justifies the need to consider the two neurotransmitters in tandem. In this work, fast-scan cyclic voltammetry (FSCV) is used to simultaneously measure the release and reuptake of histamine and serotonin, in the posterior hypothalamus and fast-scan controlled adsorption voltammetry (FSCAV) is used to measure ambient serotonin in the hippocampus of rodents following acute (peripheral injection of lipopolysaccharide) or chronic (high fat diet (45 kcal % fat) and chronic mild stress) neuroinflammation. Biochemical (for inflammation) and behavioral (for depression) analysis are correlated with neurochemical measurements. Our preliminary results allow us to hypothesize that inflammation corresponds to increased histamine release, thus increasing the inhibition of serotonin. We also find that the capacity of the selective serotonin reuptake inhibitor (SSRI) escitalopram to increase extracellular serotonin by inhibiting serotonin transporters (SERTs) is reduced in both inflammation models. Our data highlights the important role that histamine plays in modulating serotonin during inflammation and thus depression.

PP-30: Implications of palmitoylation and phosphorylation barcoding on regulation and function of the sodium hydrogen exchanger isoform 1 (NHE1)

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The sodium hydrogen exchanger isoform 1 (NHE1) is an integral membrane protein that regulates intracellular pH by exchanging an intracellular proton for an extracellular sodium ion. Multiple cellular processes are associated with NHE1 activity including coordinated cell migration, cellular proliferation, and control of cell volume. NHE1 also functions as a membrane anchoring and scaffolding protein resulting in the organization of protein complexes, formation of stress fibers, and regulation of signaling pathways within the cell. The large C-terminus (amino acids 503-815) of NHE1 extends intracellularly and functions as the major regulatory domain of the exchanger where binding partners and posttranslational modifications (PTM) influence the exchanger in both a short- and long-term manner. Elucidating mechanisms of NHE1 regulation by posttranslational modifications has been focused on contributions from the many phosphorylation sites on functional cellular outcomes. Previously, we have shown NHE1 is also regulated by palmitoylation, a reversible lipid modification in which a 16-carbon fatty acid is covalently linked to a cysteine residue via a thioester bond. We have also demonstrated that inhibition of palmitoylation in cells expressing NHE1 decreases NHE1 activity as well as stress fiber formation and cell migration, two cellular functions that are regulated through NHE1 phosphorylation. Multiple studies demonstrating a relationship between phosphorylation and palmitoylation on protein regulation have been reported. Our lab recently demonstrated a reciprocal relationship between phosphorylation and palmitoylation in regulation of the dopamine transporter. In this study, we utilized various stimuli including insulin, reduced serum, and lysophosphatidic acid (LPA), all of which are known to impact NHE1 phosphorylation and regulation through multiple kinase pathways. Here we show that modifying the phosphorylation status of NHE1 using these stimuli results in altered palmitoylation, which has been associated with altered NHE1 activity and cellular functions. This data supports the idea of a barcode hypothesis where phosphorylation and palmitoylation and other PTMs work in concert to regulate protein function including NHE1. Future studies will identify the specific palmitoylation sites and their role in the barcode hypothesis pertaining to NHE1.

PP-31: **Brain Distribution of L-Type Amino Acid Transporter 1 (LAT1)-Utilizing Prodrugs**

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L-Type amino acid transporter 1 (LAT1) is a sodium-independent heterodimeric transmembrane protein that carries bulky and neutral amino acids into the cells. It is highly expressed on the cell surfaces that require a constant amino acid supply, such as endothelial cells of blood-brain barrier (BBB), neurons and glial cells, and over-expressed in several cancer cell types. In addition to amino acids, LAT1 also carries drugs and therefore, it can have a major role in drugs' brain disposition. If a drug is not a LAT1-substrate, it can be converted as a LAT1-utilizing prodrug to improve its brain uptake and intra-brain targeting.

In this study, the protein expression ad function of LAT1 in mouse primary neurons and astrocytes as well as immortalized microglia (BV2) was evaluated. Then, the effect of Alzheimer's disease (AD; transgenic APdE9) and neuroinflammation (lipopolysaccharide (LPS)-induced) on the expression and function of LAT1 in mouse primary astrocytes was studied. Finally, the distribution of structurally diverse in-house made LAT1-utilizing neuroprotective prodrugs between neurons, astrocytes and microglia was explored. Expression of LAT1 was determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based targeted proteomics and function of LAT1 with radio-labeled LAT1-substrate, [14C]-L-leucine. The cellular uptake of LAT1-utilizing prodrugs and their parent drugs in each cell type was determined by LC-MS.

All brain cells expressed LAT1 protein within the similar range (1.2-4.2 fmol/µg crude protein) and were able to carry [14C]-L-leucine via LAT1 with a concentration-dependent manner. Moreover, the expression and function in the astrocytes was not changed due to the LPS-induced neuroinflammation or in transgenic AD-phenotype. Most importantly, all the studied prodrugs had 2-18-times higher cellular uptake in brain cells than their parent drugs. Moreover, some of the prodrugs released their parent drugs within the cells, indicating that these cells possess sufficient enzyme activity for prodrug bioconversion. Depending on the prodrug structure, selectivity between neuron-, astrocyte- and microglia- uptake was recognized. LAT1-Selectivity over other amino acid transporters and the bioconversion rate were found to have an impact on cell-type selective uptake.

In conclusion, since neurons, astrocytes and microglia express LAT1 protein, it can be utilized to increase the cellular uptake of drugs within the brain by using a LAT1-prodrug approach. This can also be achieved in cells that are already predisposed to the pathological changes of AD. Furthermore, by careful prodrug design cell-selective uptake of drug can be increased and targeting between neurons, astrocytes or microglia can be obtained, which can efficacy of neuroprotective drugs within the brain.

PP-32: **The human SLC1A5 amino acid transporter: structure/function relationships, regulatory aspects and involvement in energy metabolism.**

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The human ASCT2 transporter mediates Na- dependent antiport of neutral amino acids across plasma membrane with the function of balancing intracellular amino acid pool. Despite the acronym standing for Ala, Ser and Cys Transporter, Gln revealed to be the preferred substrate. Gln taken up by ASCT2 is used for energy purposes under proliferative conditions. Indeed, the transporter is over-expressed in most human cancers, which use Gln as the major energy source. To characterize the human transporter, the protein was produced in *P.pastoris*, purified and assayed in proteoliposomes for transport function. A functional and kinetic asymmetry has been demonstrated with certain amino acids being inwardly transported and others bi-directionally transported. Surprisingly, Cys is not a substrate but a modulator. These data were integrated by transport and post translational modification studies in intact cells. The kinetic mechanism of the transport is random simultaneous, in agreement with the trimeric structure of the protein. New insights into structure/function relationships were unveiled by site-directed mutagenesis. WT and Cys to Ala mutants were produced and reactivity towards SH reducing and oxidizing agents was investigated: transport activity increases upon treatment with reducing agent DTE, i.e., when Cys residues are reduced. Methyl-Hg, which binds to SH groups, is able to inhibit WT and seven out of eight mutants. C467A loses the sensitivity to both DTE activation and Methyl-Hg inhibition. The C467A shows a Km for Gln one order of magnitude higher than that of WT. Moreover, the C467 residue is localized in the substrate binding region of the protein, as suggested by bioinformatics on the basis of the recent 3D structure of hASCT2. Thus, C467 residue is crucial for both substrate binding and modulation of hASCT2. Indeed, physiological signaling molecules such as nitric oxide and GSH interact with the transporter through C467, modulating its transport function.

PP-33: **The Solute Carrier Transporters Interactome**

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Solute carriers (SLCs) represent the major class of metabolite and drug transporters in biological systems. However, the cellular protein complexes to which SLC proteins participate (i.e. the cellular interactome of SLCs) has been characterized only for a few members, as traditional methods to detect protein-protein interactions (PPIs) do not favor detection of SLCs. Using gel-free shotgun mass spectrometric analysis, several laboratories have in the past identified SLC38A9 as a component of the lysosomal amino acid sensing machinery that controls mTORC1. We hypothesize that elucidation of protein complexes to which SLCs participate in a systematic way should help to "deorphanize" and functionalize many poorly understood SLCs. Furthermore, it will allow to identify those SLCs that, like SLC38A9, may be linked to signaling pathways and thus play a regulatory role in integration of metabolism and cellular processes. We plan to systematically determine the protein interaction landscape of SLCs in diverse human cells lines. We will first generate genomic engineered HEK293 flp-in™ T-Rex™ cells expressing N- or C-terminally HA-TwinStrep-tagged SLC versions. HEK293 cells express ~ 200 SLCs and we will synthetize codon-optimized cDNAs sequences for these targets. We will then establish a robust one-step affinity purification coupled to mass spectrometry (AP-MS) approach, which we will next employ to obtain a first-pass characterization of the steady-state interactome around these SLCs. Once this system has been proven feasible to large-scale proteome characterization, we will expand it to five or six other cell types, allowing to cover > 80% of expressed SLCs.

PP-34: Ceefourin-1 inhibits the MRP4/ABCC4-mediated transport of several signaling molecules and attenuates integrin $\alpha_{IIb}\beta_3$ activation in platelets

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Background: The multidrug resistance protein 4 (MRP4) is a multispecific efflux transporter, known to transport second messenger molecules such as cyclic nucleotides and certain lipid mediators and is highly expressed in platelets. Recently, we identified the potent platelet-derived pro-inflammatory mediator sphingosine-1-phosphate (S1P) as an additional substrate of MRP4. The aim of the present study was to characterize the effect of the novel selective MRP4 inhibitor Ceefourin-1 on different signaling pathways in platelets, including the transport of inflammatory mediators.

Methods: In-vitro transport studies were performed using inside-out membrane vesicles from human platelets and MRP4-overexpressing SF9 cells. Platelet aggregation was determined by light transmission aggregometry after stimulation with different agonists. The exposure of CD62P as a secretion marker and the binding of FITC-labeled fibrinogen, indicating the activation status of integrin $\alpha_{IIb}\beta_3$, were measured by flow cytometry. Furthermore, a flow cytometric approach was used to quantify phosphorylation of vasodilator-stimulated phosphoprotein (VASP) at Ser^{157} and Ser^{239} as a surrogate marker for the intracellular concentrations of cAMP and cGMP, respectively. Ratiometric calcium measurements were performed in Fura 2-loaded platelets.

Results: The transport assays in isolated membrane vesicles showed a concentration-dependent inhibition of MRP4-mediated cGMP (1 µM) and thromboxane B_2 (1 µM) transport by Ceefourin-1 (IC_{50}: 6 µM and 3.5 µM). ATP-dependent transport of FITC-S1P (5 µM) was also significantly affected by Ceefourin-1 (66 ± 15% and 85 ± 22% inhibition at 50 µM Ceefourin-1 in MRP4-SF9 and human platelet vesicles, respectively; mean ± SEM). In *ex-vivo* aggregometry studies Ceefourin-1 significantly inhibited platelet aggregation by about 30 to 50% when ADP or collagen were used as activating agents, respectively. Furthermore, Ceefourin-1 significantly lowered the ADP-induced activation of integrin $\alpha_{IIb}\beta_3$ indicated by binding of FITC-fibrinogen (about 50% reduction at 50 µM Ceefourin-1 and 0.5 µM ADP). P-selectin (CD62P) surface exposure as an indicator of platelet degranulation was affected to a minor extend. Platelets incubated with Ceefourin-1 (50 µM) showed a 1.5 fold and 1.7 fold increase in PGE1 (1 µM)- and Cinaciguat (1 µM)-induced VASP phosphorylation, indicating increased cytosolic concentrations of cAMP and cGMP, respectively. Calcium influx in platelets incubated with Ceefourin-1 (50 µM) was reduced by 15%.

Conclusions: The selective MRP4 inhibitor Ceefourin-1 can interfere with integrin $\alpha_{IIb}\beta_3$ activation in platelets. This finding involves an elevated VASP phosphorylation and reduced calcium influx, which might be a result of an impaired cyclic nucleotide extrusion or sequestration due to MRP4 inhibition. Beside the potent inhibition of cyclic nucleotide transport, Ceefourin-1 can also affect the transport of pro-inflammatory mediators such as S1P.

PP-35: **A closer look at the N-terminus of LeuT**

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Background: LeuT *from Aquifex aeolicus* is a bacterial homologue of mammalian solute carrier family 6 (SLC6) neurotransmitter transporters. SLC6 transporters are of great pharmacological interests because of their crucial role in neurotransmitter clearance. These proteins are also targets of many clinically relevant drugs, including antidepressant drugs and drugs of abuse such as amphetamines, especially the latter. The amino terminus of SLC6 transporters plays an important regulatory role in their function.

Methods: High resolution X-ray crystal structures of LeuT in different conformations are available. However, these structures are not able to fully explain the dynamic nature of LeuT function during the substrate transport cycle. The aim of this project is to look at the movements of the N terminus of LeuT in a native membrane environment using spectroscopic technique called lanthanide based resonance energy transfer (LRET).

Results: We developed a LeuT variant in which we inserted a Lanthanide Binding Tag (LBT) at position 335 the extracellular site, positioned at the end of extracellular loop 4. Three LeuT mutants (K4C, H7C and A9C) were created in the background of the LeuT-LBT construct. Using LRET based distance measurements we determined the distance between the LBT bound lanthanide and the fluorophore TMR attached to the cysteines in the N-terminal of LeuT. The results did not show the expected increase in distance towards the N-terminus, as the distance to H7C was shorter that to A9C. In contrast, iodate quenching experiments of TMR fluorescence showed no difference in accessibility to KI for all three residues.

Discussion: Intramolecular distances determined by LRET suggest that the fluorophore attached to H7 could be oriented towards the inner vestibule, while the KI based accessibility experiments displays a contrasting picture. In order to elucidate the dynamics of the N-terminus and reinforce our findings, I am further planning to stabilize the transporter in an outward open conformation using tryptophan, and as an alternative strategy, stabilize LeuT-LBT in the inward-facing and an outward-facing conformation using the R5A or the R30A mutants.

PP-36: **Structure-function of human ABCG2: Insights for a novel drug transport mechanism**

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Multidrug resistance (MDR) is a major issue of chemotherapeutic treatment in human cancer. Drug efflux via ATP-binding cassette (ABC) transporters is one of the key mechanisms that reduce drug accumulation and protect tumor cells from cytotoxicity that causes an aggressive metastasis and failure of treatment. Human ABCG2 or Breast Cancer Resistance Protein (BCRP) plays a vital role in physiological detoxification, involved in anticancer multidrug resistance and related to genetic diseases of gout. However, despite numerous recent x-ray and cryo-EM structures that became available for several ABC transporters, the precise mechanism of function is still unclear. In this study, we extensively perform the molecular-genetic study and structure-function analysis with experimental verifications to provide the strong evidence that support a distinct fold and novel mechanistic regulation of catalytic cycle and drug transport. A unique arrangement at transmission interface of ABCG2 suggests a pivotal role of NBD-TMD interaction to control conformational switch. ABCG2 structure at outward-facing configuration shows a large central cavity thus explains the extremely broad substrate specificity and drug recognition whereas a polar relay in the inner core is important for structural rigidity. Furthermore, the extracellular-membrane interface region is imperative for drug extrusion. All extracellular loops and re-entry helix are involved in ABCG2 biogenesis. Interestingly, leucine valve at the top of central cavity is crucial for substrate translocation to the upper cavity before drug release through a polar roof at the extracellular region. The perception of ABCG2 structure and drug transport mechanism provides valuable insights that could be a beneficial advantage for the development of novel pharmacological approaches to improve specific ABCG2 substrates as well as inhibitors for cancer medication. These novel mechanistic data open new opportunities to therapeutically target ABCG2 in the context of related diseases.

PP-37: **Structure-activity relationship and molecular determinants of betaine/GABA transporter 1 (BGT1) inhibitors**

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As a member of the GABAergic system, the GABA transporters (GATs) play a critical role in the regulation and termination of the GABA-mediated signaling as they function as key proteins in neurotransmitter uptake. We have previously identified-amino-1,4,5,6-tetrahydropyrimidine-5-carboxylic acid (ATPCA) as the most potent substrate-inhibitor of the betaine/GABA transporter 1 (BGT1) reported to date. In order to characterize the molecular basis of GABA-transporter subtype selectivity, a series of ATPCA analogues were synthesized and pharmacologically characterized in radioligand-based uptake assays at the four human GABA transporters (hGATs) recombinantly expressed in mammalian cells. Overall, the analogues retained subtype-selectivity for hBGT1, though with lower inhibitory activities compared to ATPCA. Further characterization of five BGT1-active analogues in a fluorescence-based FMP assay revealed that the compounds are substrates for hBGT1, suggesting that they interact with the orthosteric site of the transporter. In silico-guided mutagenesis experiments showed that the non-conserved residues Q299 and E52 in hBGT1 potentially contribute to the subtype-selectivity of ATPCA and its analogues. Computational docking studies and molecular dynamics simulations suggested that these residues form stable hydrogen bonds with the guanidine or amidine moieties of ATPCA and its derivatives. Overall, this study provides new insights into the molecular interactions governing the subtype-selectivity of BGT1 substrate-inhibitors.

PP-38: **Mad2, a novel player in clathrin-mediated endocytosis, interacts with monoamine transporters.**

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Monoamine transporters conduct the reuptake of serotonin (SERT), dopamine (DAT) and norepinephrine (NET) following neurotransmission. Surface levels and subcellular localization of transporter proteins can be regulated by clathrin-mediated endocytosis (CME), during which cargo proteins internalize as part of surface-derived membrane vesicles. This process requires adaptor protein 2 (AP2), which links cytosolic domains of cargo proteins to a cage of clathrin proteins. This leads to the formation of intracellular clathrin-coated vesicles. It is currently unknown how monoamine transporters connect to clathrin, as their intracellular domains do not provide any known interaction motif for AP2 binding. Though, the carboxy-terminus of the transporter was shown to play a central role for its internalization.

Recent work shows that the insulin receptor (IR) interacts with AP2 via a heterodimer of two mitotic spindle assembly checkpoint (SAC) proteins: MAD2 and Bubr1. A classical MAD2 interacting motif (MIM) in the IR C-terminus is crucial for AP2 recruitment.

Inspection of monoamine transporter C-termini reveals putative MIMs, similar to those found in other MAD2 interacting proteins (IR; CDC20; Mad1). Considering this similarity and the acknowledged but opaque role of the transporter C-terminus for endocytosis, it is reasonable to hypothesize that MAD2 initiates clathrin-mediated endocytosis of neurotransmitter transporters.

A combination of biochemical methods was used to study a putative SERT – MAD2 interaction. Consequences of MAD2 depletion on SERT surface expression and subcellular localization were investigated using siRNA mediated knock-down.

The conducted experiments clearly show an interaction between MAD2 and monoamine transporters at the cellular surface. This interaction is dependent on the MAD2 interacting motif in the transporter C-terminus. Interestingly, the cognate transporter GAT1 misses MAD2 interaction. MAD2 depletion in YFP-SERT expressing cells causes significant increase of SERT surface expression and differential SERT-glycosylation. Furthermore, MAD2 knock-down depletes intracellular membrane compartments from YFP-SERT, indicating disturbed endocytosis.

These results suggest a role for MAD2 during endocytosis of monoamine transporters. Since MAD2 shows marked expression in the brain, it is plausible to assume that the investigated interaction also occurs in a native biological system. Hence, this work could provide an answer to the puzzling question of the interplay between monoamine transporters and the endocytic machinery.

PP-39: **A rare DAT variant perturbs behaviour in mice in a gene dose-dependent manner: Hyperactivity is the least of their problems.**

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Dysregulation of dopamine signalling in the brain has been associated with psychiatric disorders, such as ADHD, autism, schizophrenia, and with movement disorders such as Parkinson´s disease. Missense mutations in the dopamine transporter (DAT) can lead to dysfunctional dopamine homeostasis and cause not only a cellular physiopathology but also noticeable phenotypes in mice and humans. Several rare coding variants have been discovered in the *SLC6A3* gene encoding DAT in ADHD patients and more in other patients with psychiatric disorders.

We have identified a male patient who was compound heterozygous for $hDAT^{I312F}$ and $hDAT^{D421N}$ mutations and diagnosed with atypical Parkinsonism and ADHD. He suffered from intellectual impairment, learning difficulties, lack of impulse control and tremors progressing in severity and extensiveness. The two residues I312 and D421 in are highly conserved across species and part of the membrane-spanning domains of DAT. Both mutations have been characterised in-vitro and ex-vivo and show notable decrease of transport capacity. The D421N mutation decreases uptake capacity to 10% of the WT in-vitro and ex-vivo.

Subsequently, mice were generated to carry one DAT mutation (corresponding I311F or D420N point mutation) and bred in a heterozygous manner among the same line, but also crossed - to create three mouse lines in total. Resulting homozygous knock-ins and compound heterozygous mice of the mouse strains display different behavioural characteristics.

Here, we show results from an ongoing study focusing on the characterization of a mouse model carrying the corresponding mutation $mDAT^{D420N}$ found in the patient to understand the impact of the single mutation in the compound heterozygous genotype. The physical performance of homozygous knock-in (KI), their heterozygous (DN HET) and their wild type (WT) littermates has been examined using a battery of classical behavioural tasks. This includes movement tracking in the open field and in the elevated plus maze, evaluation of motor skills, such as motor coordination, motor learning, grasping and climbing skills at young adult age and 1 year of age. The mice were further tested for abnormal clasping. Overall, the homozygous knock-in mice are smaller than their littermates, display remarkable vertical stereotypic behaviour and a pronounced novelty-driven hyperactivity. Clasping as shown previously in various models for Parkinsonism was observed. Males appear to exhibit pronounced aggression related to dominance beyond their C57BL/6J background.

PP-40: **SLC6A14 – an amino acid transporter $B^{0,+}$, interacts exclusively with SEC24C as a cargo recognizing COPII element during exit from endoplasmic reticulum**

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SLC6A14 , a plasma membrane amino acid transporter $B^{0,+}$ ($ATB^{0,+}$), encoded by the *SLC6A14* gene, is a Na/Cl dependent transporter specific for neutral and basic amino acids. It is up-regulated in several types of malicious cancers. In the first step of trafficking to the plasma membrane, it has to leave the endoplasmic reticulum (ER) and to interact with a cargo recognizing protein SEC24 within Coatomer II (COPII) complex. We studied trafficking of ratSLC6A14 in a heterologous expression system in HEK293 cells. Western blot analyses, deglycosylation experiments and immunofluorescence studies demonstrated that the substantial amount of fully glycosylated SLC6A14 appears at the cell surface after 48h. Cell surface biotinylation studies showed a decrease in SLC6A14 presence in plasma membrane by dominant negative mutants of SAR1, a GTPase, whose activity triggers the formation of the COPII complex. Out of four SEC24 paralogues SLC6A14 co-precipitated exclusively with SEC24C isoform and a direct interaction with SEC24C was confirmed by proximity ligation assay. Co-localization of endogenous SLC6A14 with SEC24C was also observed in MCF-7 breast cancer cells. Contrary to the endogenous transporter, part of the overexpressed $ATB^{0,+}$ is directed to proteolysis, a process significantly reversed by a proteasome inhibitor bortezomib. Co-transfection with a SEC24C dominant negative mutant attenuated $ATB^{0,+}$ expression at the plasma membrane, due to proteolytic degradation. Analysis of SLC6A14 amino acid sequence and detected specificity for SEC24C confirms a hypothesis proposed for a neurotransmitter transporters branch of SLC6 family that a hydrophilic residue at +2 position downstream of the ER export “RI” motif determines interaction with C isoform of SEC24 proteins and promotes further trafficking to Golgi and plasma membrane. Moreover, there is an equilibrium between export from ER and degradation mechanisms in case of overexpressed transporter.

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PP-41: **The iPSC-derived dopamine neurons from a healthy subject and a patient with α-synuclein triplication reveal novel therapeutic target**

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Parkinson's disease (PD) is characterized by progressive loss of dopamine (DA) neurons in the substantia nigra (SN-A9). α-synuclein has been genetically implicated in familial and sporadic PD and is associated with PD susceptibility, progression and pathology. A cardinal feature of neurons that separates them from nearly all other cell types is their excitability. Multiple channels and transporters regulate the excitability of DA neurons. DA neurons are uniquely defined by expression of Dopamine Transporter (DAT), D2 autoreceptor and GIRK, where they govern the excitability of DA neurons, and thus DA neurotransmission. To determine the impact of α-synuclein on the activity of DA neurons, we differentiated induced pluripotent stem cells (iPSC) from PD patients with α-synuclein triplication and the healthy sibling. Two sets of iPSCs used in our studies: iPSCs with triplication of α-synuclein (AST), and iPSCs with normal α-synuclein (NAS) levels. The A9 human-like DA neurons were differentiated and identified by expression of Tyrosine Hydroxylase (TH), GIRK2, VMAT2, DAT, Nurr1, FOXA2, as phenotypic markers for DA neurons. The expression of α-synuclein and Parkin, two PD-associated proteins were higher in the AST-derived human-like DA neurons as compared to the NAS-derived human-like DA neurons. The AST neurons exhibited significantly larger soma, short neurites and lower D2R and GIRK expression. Importantly, our differentiation protocol resulted in spontaneously firing human-like DA neurons. Whole-cell voltage-clamp recordings revealed no difference in the overall activity of sodium and potassium channels in the AST and NAS DA neurons. Consistent with our previous report in mice DA neurons (Lin et al., 2016), the majority of NAS-derived DA neurons exhibited a mixture of single spikes and small burst activity with an underlying "pacemaker-like" periodicity. Whereas, ~90% of AST-derived DA neurons exhibited a unique firing pattern of spontaneous firing activity with a pause between subsequent broadbrimmed burst clusters on a high depolarized plateau (up state). We found, in the AST-derived DA neurons, activation of D2R or GIRK channels reduced the amplitude and the width of the up state and suppressed the firing frequency, suggesting α-synuclein triplication disrupts DA transmission via dysregulation of D2R and GIRK channels.

PP-42: **Allosteric properties of a putative third sodium site in the glycine transporter GlyT2**

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The removal of neurotransmitter from glycine-mediated synapses depends on two high-affinity plasma membrane glycine transporters (GlyTs) that control neurotransmitter availability by sodium-driven uptake. GlyT1 is the main regulator of glycine levels in the synaptic cleft, whereas the neuronal GlyT2 is involved in the recycling of synaptic glycine and also supplies neurotransmitter for maintaining the quantal glycine content inside the synaptic vesicles. GlyT1 and GlyT2 differ in sodium:glycine symport stoichiometry, showing GlyT1 a 2:1 and GlyT2 a 3:1 sodium:glycine coupling. The GlyTs contain two conserved Na^+ sites: Na1 and Na2. The location of the third sodium site (Na3) in GlyT2 remains unknown, although Glu650 has been involved in the coordination. Here we have used comparative molecular dynamics simulations of a GlyT2 model constructed by homology to the crystalized dopamine transporter from *Drosophila melanogaster* by placing the Na3 ion at two different locations. By combination of in silico and experimental data obtained by biochemical and electrophysiological analysis of GlyTs mutants, we provide evidences suggesting the GlyT2 third sodium ion is located within a region with robust allosteric properties involved in cation-specific sensitivity. Mutagenesis of a residue present in the putative third sodium site in GlyT2 by the corresponding amino acid in GlyT1 reduced the charge-to-flux ratio to the level of GlyT1 without producing transport uncoupling. Determinations of the reversal potential for sodium suggest two or one charge coupling for the wild-type and the mutant. The differential behavior of equivalent GlyT1 mutations sustains specific allosteric properties of the putative Na3 site in GlyT2.

PP-43: **Phase I metabolites of methylenedioxy-substituted stimulants interact with human monoamine transporters**

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Methylenedioxy-substituted stimulants are among the most popular illicit drugs of abuse, exerting stimulant or entactogenic effects. Enzymatic dealkylation or opening of the methylenedioxy ring gives rise to metabolites that may potentially be pharmacologically active. Previous studies in rats indicate that several metabolites of 3,4-methylenedioxymethamphetmine (MDMA), 3,4-methylenedioxymethcathinone (methylone), and 3,4-methylenedioxypyrovalerone (MDPV) interact with monoaminergic systems and the aim of the current study was therefore to investigate the interactions of these metabolites with human monoamine transporters.

We treated human transporter-transfected human embryonic kidney 293 cells with various concentrations of the metabolites and parent compounds to assess the inhibition of monoamine uptake by transporters for norepinephrine (NET), dopamine (DAT), and serotonin (SERT). As transporter substrates, we used ^{3}H-labeled norepinephrine, dopamine, or serotonin.

N-demethylation of MDMA only slightly altered the monoamine uptake inhibition profile, whereas *N*-demethylation of methylone decreased the overall transporter inhibition potency. *O*-demethylenation of MDMA, methylone, and MDPV resulted in catechol metabolites that mostly maintained the norepinephrine, dopamine, and serotonin uptake inhibition potency of the parent compounds. *O*-methylation of the catechol metabolites significantly decreased the norepinephrine uptake inhibition, resulting in metabolites lacking significant stimulant properties.
To conclude, several metabolites of MDMA, methylone, and MDPV interact with human monoamine transporters at pharmacologically relevant concentrations. The *N*-dealkylated metabolites are found in plasma in unconjugated form and therefore potentially play a pharmacological relevant role. In contrast, the high proportion of conjugates observed for *O*-demethylenated metabolites suggests that these metabolites make only minor contributions to the pharmacological effects.

PP-44: **Interactions of Drugs with Organic Solute Transporter Alpha/Beta (OSTα/β): Influence of Preincubation and Physicochemical Properties of Compounds on OSTα/β Inhibition**

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The interaction of drugs with the organic solute transporter alpha/beta (OSTα/β) is understudied even though OSTα/β is expressed in multiple human tissues including the liver, kidneys and intestines. In the present study, an *in vitro* method for identification of OSTα/β inhibitors was developed, and the extent of inhibition by seventy-seven structurally diverse compounds or fixed-dose combinations towards OSTα/β was evaluated. These compounds were derived from a dataset of 190 cholestatic drug-induced liver injury cases, and were supplemented with classical hepatotoxic compounds, previously identified OSTα/β inhibitors, and cholestatic bile acids. OSTα/β-mediated substrate uptake was measured in OSTα/β-overexpressing Flp-In 293 cells in the absence or presence of the putative inhibitors. Optimal study conditions were determined by comparing the extent of inhibition of known inhibitors when they were either co-incubated with the substrate or preincubated before substrate administration. Data indicated that a 10-min preincubation produced a greater extent of inhibition than co-incubation. Using the 10-min preincubation approach, our results revealed that ethinyl estradiol, norgestimate, atorvastatin, troglitazone sulfate, spironolactone, fidaxomicin, and a fixed-dose combination, ethinyl estradiol-norgestimate (100 µM) inhibited OSTα/β-mediated dehydroepiandrosterone sulfate (DHEAS, 4 µM) uptake. Also, an abundant bile acid, glycochenodeoxycholate (100 µM) decreased cellular uptake of DHEAS. OSTα/β inhibition by ethinyl estradiol, norgestimate, atorvastatin, troglitazone sulfate, and fidaxomicin was further evaluated by determining the half-maximal inhibitory concentration (IC_{50}) for these compounds using DHEAS (4 µM) as an OSTα/β probe substrate. In addition, the inhibitory effect of ethinyl estradiol and fidaxomicin was confirmed with digoxin (1 µM) as an OSTα/β probe substrate. There was no clear correlation between the physicochemical properties of compounds and the degree of OSTα/β inhibition. Furthermore, the hepatotoxicity ranking of the compounds did not correlate with the extent of OSTα/β inhibition. This study utilizes a novel *in vitro* method for direct assessment of drug interactions with OSTα/β. Our data provides evidence that several drugs, some of which are associated with cholestatic drug-induced liver injury, may disturb the function of the OSTα/β transporter.

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PP-45: **Sulfenamide Derivatives Can Improve Oct-Mediated Cellular Uptake Of Metformin**

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Numerous studies have shown that metformin is a substrate for organic cation transporters (OCTs), which determines its oral absorption, distribution and elimination (hepatic uptake and renal excretion) as well as biochemical effects in humans. Since metformin has a relatively low bioavailability and high variations in pharmacodynamic response between patients, several metformin sulfenamide and sulfonamide derivatives were synthesized [(1),(2)]. The aim was to study cellular uptake and the role of OCTs in metformin and its derivatives transport, and if sulfenamide derivatives of metformin exhibit better cellular uptake rate than metformin itself.

The affinity of metformin and sulfenamides to bind to OCTs was examined by the establishment of [^{14}C]-choline uptake in MCF-7 and MDA-MB-231 human breast cancer cell lines. Furthermore, the cellular uptake of metformin and its derivatives into these cells was evaluated with high performance liquid chromatography (HPLC) method. For a better understanding of transport mechanisms utilized in cellular uptake of metformin derivatives the studies in the presence of dysopiramide, an OCT1 inhibitor, were conducted.

The maximal inhibition of [^{14}C]-choline uptake was reported for the highest concentration of metformin (2400 µmol/L); 34.3% and 29.9% for MCF-7 and MDA-231 cells, respectively. Sulfenamide derivatives were characterized by greater ability to bind to OCTs than metformin. The half maximal inhibitory concentration (IC_{50}) value in MCF-7 for derivative with *n*-octyl chain was 236.1 ± 1.28 µmol/L. This indicates that the compound has the highest affinity to bind to OCTs as well as potency to inhibit the transporters in MCF-7 cells. In addition similar properties were found for this derivative in MDA-231 cell line (IC_{50} = 217.4 ± 1.33 µmol/L).

The highest uptake in MCF-7 cells was reported for cyclohexyl and *n*-butyl derivatives, which at concentration of 800 µmol/L reached velocity of over 1.2 nmol/min/mg of proteins. This was approximately 11-fold higher than with metformin. Similarly, in the case of MDA-231 cells the highest uptake was found to be with cyclohexyl derivative (0.956 ± 0.065 nmol/min/mg of proteins). In addition, *n*-octyl derivative was also characterized by better uptake than metformin, in MCF-7 and MDA-231 reaching 1.02 ± 0.13 and 0.79 ± 0.08 nmol/min/mg of proteins, respectively. Eadie-Hofstee analysis of *n*-octyl sulfenamide uptake showed two OCTs transporters engaged in cellular uptake of the derivative in both cell lines. Both transporters in MCF-7 cells appeared to be more efficient than in MDA-231 cells, and *n*-octyl derivative possessed higher affinity towards the transporters in MCF-7 cells as the respective K_m values were lower.

In summary, chemical transformation of metformin into sulfenamides differing in the length of alkyl chain or presence of saturated hexyl ring contributed to the obtaining of better substrates for OCTs, and higher cellular uptake in MCF-7 and MDA-231 cells. The alkyl chain introduced to the sulfenamides contributed to the selelectivity between OCT1 and OCT3 and transport efficacy.

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PP-46: **The combination of alcohol and cocaine affects monoamine transporters of the SLC-6 family and organic cation transporter 3**

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A plethora of psychoactive substances hijack the natural reward-circuitry and serve as chemical replacements for natural rewards. Cocaine, a widely abused psychostimulant, inhibits the high-affinity transporters for dopamine (DAT), serotonin (SERT) and norepinephrine (NET), thus leads to elevated concentrations of these monoamines in the extracellular space. Ethanol-intake has been associated with profound elevations in extracellular serotonin. However, ethanol does not affect SERT-function. Here we show that ethanol inhibits uptake by the organic cation transporter 3 (OCT3), an additional re-uptake mechanism for monoamines. Real-time measurements using fluorescent tracers revealed that ethanol did not perturb SERT-mediated uptake, whereas profound effects were observed on OCT3 at physiologically relevant concentrations. Similarly, ethanol inhibited uptake of [^{3}H]MPP+ into OCT3 expressing HEK293 cells, but not mock transfected cells. These findings were confirmed using *in vivo* chronoamperometry, with ethanol inhibiting 5-HT clearance in CA3 region of hippocampus in OCT3 wild-type but not in OCT3-deficient mice. These findings not only add to our general understanding of the mechanism of action of ethanol, but may also provide a molecular explanation for one of the most prevalent drug-combinations. While cocaine disrupts the function of DAT, NET and SERT, the effects of ethanol on the cocaine-insensitive OCT3 boost cocaine-induced increase in extracellular monoamines.

PP-47: **Examination of ABCG2 mutations found in gout patients**

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ABCG2 is a member of the ABC-type multidrug transporter protein family and plays an important role in the protection of our cells by exporting toxic substances. In our research we focused on the role of ABCG2 in gout. Increased serum uric acid level (hyperuricaemia) is the precondition of the development of gout and ABCG2 actively transports urate among many other molecules. The ABCG2 protein is expressed in the proximal tubules of the kidney, in the intestine, and in several other tissues. ABCG2 maintains the balance of uric acid secretion and absorption, which, in the case of a dysfunctioning ABCG2 protein, may shift towards absorption, and lead to hyperuricaemia and gout. According to our previous study, there are ABCG2 polymorphisms and mutations that are more common among gout patients. By further examining these mutant variants we suggest that in these cases the disfunction or lower expression level of ABCG2 may play a role in the development of the disease. It is important to characterize these clinically relevant mutations, as a directed genetic analysis can help to find the best personal treatment for the patients. It is important to mention that ABCG2 is also present in the blood-brain barrier and has many substrates that are commonly used drugs in medicine. By knowing that a patient has a dysfunctional variant of the ABCG2 protein, a personalised treatment with an ABCG2-substrate drug should be used. A recent study showed the presence of as yet unknown missense ABCG2 mutations in gout patients, while no information on the cellular effects of these mutations were presented. Therefore, we decided to examine the function and expression level of these ABCG2 variants in a HEK 293H cell line, transfected with vectors containing the mutant ABCG2 cDNA. Our data show greatly variable functional effects of these previously uncharacterized mutant variants.

PP-48: ***para*-substituted methcathinones as selective and unselective inhibitors of human dopamine and serotonin transporter**

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Methcathinone (MCAT) is a compound belonging to the class of cathinones and it is targeting monoamine transporter including DAT and SERT. Despite the importance of DAT and SERT as drug targets in several neurological disorders, the key factors underlying the selectivity profile of their inhibitors is still poorly understood. Recent findings from rat synaptosomes suggested that increasing the volume of the para- substituent of MCAT results in a swap of the selectivity between human DAT and SERT (1). Docking studies hint towards Ser149 in DAT and Ala169 in SERT as key residues involved in the difference of activity between DAT and SERT (2). The aims of the present biochemical and pharmacological study are to understand (i) which chemical properties (e.g. volume, polarity or lipophilicity) of the para-substituent influence the selectivity profile of MCAT between DAT and SERT, and (ii) whether Ser149 in DAT and Ala169 in SERT can be experimentally verified as key residues. Hence, we combined in silico-driven synthesis, mutagenesis and radio-tracer flux assays in HEK293 cells expressing the human DAT and SERT wild type and respective mutants. We found that only MCAT and CF_3-MCAT showed high selectivity, 200 fold for DAT/SERT - and 25 fold for SERT/DAT, respectively. This suggests that the high selectivity achieved is determined rather by specific features of these compounds than by the volume of the para-substituent. Accordingly, we were not able to find any correlationship between the selectivity profile of the tested 4MCATs with either volume, polarity and lipophilicity parameters. Next, based on our results, we tested the hypothesis that the two amino acids in SERT and DAT do not suffice to explain the selectivity between SERT and DAT. We have tested the para-substituted methcathinones in the swapping mutations DAT Ser149Ala and SERT Ala169Ser and in line with our hypothesis, these mutations did not revert the selectivity profile found in the wild-type transporters. Our findings rather suggest that the SERT selectivity achieved by the introduction of the CF_3- group in the para-position of MCAT is not dependent on the volume of the para-substituent but on specific chemical features of the fluorine atoms which may influence the on and off rate of the MCAT moiety on DAT and SERT.

PP-49: **Dual and direction-selective mechanisms of phosphate transport by the vesicular glutamate transporter**

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Vesicular glutamate transporters (VGLUTs) fill synaptic vesicles with glutamate and are thus essential for glutamatergic neurotransmission. However, VGLUTs were originally discovered as members of a transporter subfamily specific for inorganic phosphate (P_i). It is still unclear how VGLUTs accommodate glutamate transport coupled to an electrochemical proton gradient $\Delta\mu H^+$ with inversely directed P_i transport coupled to the Na^+-gradient and the membrane potential. Using both functional reconstitution and heterologous expression, we now show that VGLUT transports glutamate and P_i using a single substrate binding site but different coupling to cation gradients. When facing the cytoplasm, both ions are transported into synaptic vesicles in a $\Delta\mu H^{+-}$-dependent fashion, with glutamate preferred over P_i. When facing the extracellular space, P_i is transported in a Na^+-coupled manner, with glutamate competing for binding but at lower affinity. We conclude that VGLUTs have dual functions in both vesicle transmitter loading and P_i homeostasis within glutamatergic neurons.

PP-50: **Systematic genetic mapping of necroptosis identifies SLC39A7 as modulator of death receptor trafficking**

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Regulation of immune cell homeostasis by programmed cell death is key to a functioning immune system. The advent of scalable somatic cell genetic technologies creates the opportunity to functionally map all elements of these fundamental processes thereby identifying potential disease-relevant components. We investigated the genetic basis underlying necroptotic cell death by performing a complementary set of loss- and gain-of-function genetic screens. We established Fas Associated Via Death Domain (FADD)-deficient haploid human KBM7 cells, that specifically and efficiently entered necroptotic cell death after a single treatment with either TNF or the SMAC mimetic compound birinapant. A series of unbiased gene-trap screens identified key signaling mediators, such as TNFR1, RIPK1, RIPK3, and MLKL. Among the novel components, we focused on Zinc transporter SLC39A7 that led to necroptosis resistance by affecting TNF receptor trafficking and ER homeostasis. Orthogonal, solute carrier (SLC)-focused CRISPR/Cas9 library-based genetic screens revealed the exquisite specificity of SLC39A7 within ~ 400 SLC genes for TNFR1 /FAS but not TRAIL-R1 responses. The established novel cellular model also allowed the genome-wide gain-of-function screen for necroptosis resistance, via the CRISPR/Cas9 synergistic activation mediator approach. Among these, we prominently found inhibitor of apoptosis (cIAP)1 and cIAP2, but also TNFAIP3-interacting protein 1 (TNIP1). TNIP1 overexpression prevented pathway activation in a ubiquitin-binding dependent manner. Within the limits of the cellular-based readout, the gain- and loss-of-function screens described here together provide a global chart of necroptosis and death receptor signalling genes, prompting investigation of their individual contribution and potential role in pathological conditions associated with disrupted immune cell homeostasis.

PP-51: **Structural dissection of 13α-estrones based on interaction with human Organic Anion-Transporting Polypeptide, OATP2B1**

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Human organic anion-transporting polypeptides (OATPs) encoded by the SLCO genes are membrane proteins that mediate the cellular uptake of large organic molecules. The endogenous substrates of OATPs are hormones, bilirubin, prostaglandins and bile acids. Additionally, several OATPs also recognize clinically applied drugs, including statins, antivirals, antihistamines and chemotherapeutic agents. Several OATPs are overexpressed in tumors of the breast, colon, lung or pancreas. Although their exact role in tumor development and therapy response is still unclear, OATPs are potential targets of anti¬cancer therapy.

One of the main strategies to fight hormone dependent tumors is the inhibition of their hormone uptake, synthesis or metabolism. To prevent the estrogen formation and proliferation of hormone dependent cancer cells, inhibiting the 17β-HSD1 (17β-hydroxysteroid dehydrogenase 1) e.g. by 13α-estrones is a promising strategy.

OATP2B1 is a multispecific transporter that is expressed ubiquitously in the human body, and is also overexpressed in various tumors (e.g. breast and prostate cancer). Being a steroid hormone transporter OATP2B1 is a good candidate to promote uptake of steroid¬based 17β-HSD1 inhibitors.

The purpose of our investigation was to unfold the interaction between 13α-estrone derivatives and OATP2B1. For this aim, we characterized OATP2B1 steroid interaction in several ways. We measured the effect of the compounds on OATP2B1 mediated transport of the fluorescent test substrate. Additionally, we measured direct transport of the compounds by OATP2B1, and we also determined the cytotoxic effect in parental and OATP2B1 overexpressing cells. We identified several potent inhibitors and potential substrates of OATP2B1. Moreover, several 13α-estrone derivatives proved to be more toxic to A431 cells overexpressing OATP2B1. Based on the interaction profile of these steroids we could define structural elements important for OATP2B1 interaction and selective cytotoxicity.

PP-52: **Association of SUR1/*ABCC8* with the K_{ATP} channel pore subunit, Kir6.2/*KCNJ11*, alters the receptor's allosteric properties.**

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K_{ATP} channels, $(SUR1/Kir6.2)_4$, are metabolite sensors that couple the membrane potential of neuroendocrine cells to metabolism. ATP has dual effects: binding to the Kir6.2/*KCNJ11* pore closes channels, while interaction with the SUR1/*ABCC8* regulatory subunits oppose this inhibition. Understanding the allosteric protein network(s) linking conformational changes in SUR1 to channel openings requires, at minimum, relating structural data on ABC proteins to channel activity. The interaction of the antagonist, [^{3}H] glibenclamide ([^{3}H]GBM), with SUR1, expressed with or without Kir6.2, was used as a reporter to identify specific ABC conformers with channel activity. ATP binding, without hydrolysis, i.e., the 'power stroke' of an ABC transporter, was enough to shift SUR1 monomers to states with higher K_Ds for [^{3}H]GBM and lower K_Ds for an agonist, diazoxide [1]. Inward-facing receptor conformers are stabilized ~7-fold by GBM binding, consistent with cryoEM structures of GBM-stabilized, closed K_{ATP} channels with SUR1 in inward-facing states [2, 3]. Thus ATP-bound SUR1 in outward-facing states should activate channel openings. This was tested using K_{ATP} channels whose $Kir6.2_{G334D}$ subunits are poorly inhibited by ATP and whose regulatory $SUR1_{Q1179R}$ or $SUR1_{E1507Q}$ subunits have lower K_Ds for ATP versus wildtype. Both channels are activated by ATP^{4-} or GTP^{4-} without Mg^{2+} needed for hydrolysis. $SUR1_{Q1179R}$ and $SUR1_{E1507Q}$ are neonatal diabetes (ND) mutations and the E1507Q substitution lacks the catalytic E needed for efficient hydrolysis. The results indicate ATP-binding to SUR1 at physiologic nucleotide concentrations will bias channels toward open states [1]. Thus *ABCC8* mutations that lower K_Ds for nucleotides will increase channel activity and produce ND, while higher K_Ds for ATP reduce openings and cause congenital hyperinsulinism [4].

Association with wtKir6.2 puts constraints on SUR1, lowering the K_D for GBM and significantly attenuating the negative allosteric interaction(s) between the ATP and GBM binding sites. The structural 'pathway' between the ATP and GBM sites is undefined. The ND Q1179R mutation introduces a positive charge near the top of transmembrane domain (TMD) helix 15 within 20 nm of the GBM binding site. The Q→R substitution reduces the estimated K_D for ATP and restores the coupling between sites suggesting the 'swapped' TM helixes, 15 and 16, that link TMD2 with NBD1 are part of a pathway coupling ATP and GBM binding in SUR1.

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PP-53: **Can ABC transporters with a degenerate NBS stay active by avoiding nucleotide occlusion?**

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A significant number of ABC exporters carry a degenerate nucleotide binding site (NBS), which shows a strongly reduced ATPase activity. The hallmark of the degenerate NBS, which is typically NBS1, is the substitution of the catalytic glutamate in the Walker B motif. The multidrug transporter ABCB1 (P-glycoprotein) and the bile salt export pump ABCB11 (BSEP) are both full-length members of the ABCB subfamily and share 49% sequence identity, but importantly, ABCB1 has two canonical NBSs, while in ABCB11 one is degenerate. A comparison of their NBD interfaces reveals that the entire interface differs in only four residues, which are all located in NBS1. The divergent residues are the catalytic glutamate of the Walker B motif of NBD1, the Q-loop residue S474 and the residues G1178 and Q1180 in the signature sequence of ABCB1. The respective residues in ABCB11 are M584, E502, R1221, and E1223. When introducing the analogous catalytic glutamate mutation (E556M) into ABCB1, the transporter becomes transport incompetent and conformationally restrained. However, when including the additional three mutations, the transporter regains the ability to hydrolyse ATP and undergo conformational changes also in the TMDs, which are reminiscent of wild type ABCB1. Simulations showed that the single mutant is conformationally locked and occludes ATP similar to WT ABCB1. The prevention of hydrolysis by the E556M mutation therefore leads to an arrest of the transport cycle. In contrast, the quadruple mutant changes the mode of ATP binding by altering the geometry of the NBD dimer and weakening the interactions between ATP and the NBS. Thus, the non-canonical NBS1, as present in ABCB11, has the ability to escape the conformationally locked state of the single catalytic glutamate mutant (E556M) by avoiding ATP occlusion.

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PP-54: **FLT3 inhibitors midostaurin, gilteritinib and FLX925 and their role in ABCB1- and ABCG2-mediated multidrug resistance**

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The novel FMS-like tyrosine kinase 3 (FLT3) inhibitors - midostaurin, gilteritinib, and FLX925, exhibit promising activity against acute myeloid leukaemia (AML) *in vitro* and *in vivo*. The presence of ABC efflux membrane transporters, which play an important role in multidrug resistance phenomenon (MDR), was confirmed in majority of haematological malignancies including AML and are currently recognized as negative prognosis biomarkers. Therefore, this study focuses on the potential of the abovementioned FLT3 inhibitors to interact with ABCB1 and ABCG2. Inhibitors and substrates of these transporters might modulate the MDR and contribute to pharmacokinetic drug-drug interactions (DDIs) possibly resulting in an altered drug disposition during pharmacotherapy.

Intracellular accumulation assays were employed in order to evaluate the potential inhibitory effect of the studied compounds towards the ABC transporters in the human myeloblastic HL60 cell line and its ABCB1-, ABCG2-, and ABCC1-overexpressing resistant variants. To assess, whether the transporters could play a role in cellular resistance to studied drugs, XTT cell proliferation assays were carried out in the same cellular model. The capability of these drugs to induce *ABCB1* and *ABCG2* was evaluated in intestinal LS174T and Caco-2 cell lines employing qRT-PCR.

We found that midostaurin, gilteritinib and FLX925 are able to increase the intracellular concentrations of daunorubicin and mitoxantrone and thus show inhibitory activity towards ABCB1 and ABCG2, respectively, at clinically relevant concentrations. Moreover, ABCG2 transporter is likely to confer resistance to midostaurin. Interestingly, induction studies indicate that all studied drugs are able to induce *ABCB1* transporter on mRNA level.

In summary, our results suggest that midostaurin, gilteritinib, and FLX925 can modulate multidrug resistance by inhibiting ABCB1 and ABCG2 transporters and simultaneously are capable to induce ABCB1. Moreover, ABCG2 might be causative of midostaurin resistance.

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PP-55: **ABCB6 resides in melanosomes and regulates early steps of melanogenesis required for PMEL amyloid matrix formation.**

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Genetically inheritable pigmentation defects provide a unique opportunity to reveal the function of proteins contributing to melanogenesis. Dyschromatosis universalis hereditaria (DUH) is a rare pigmentary genodermatosis associated with mutations in the *ABCB6* gene. Here we use optical and electron microscopy imaging combined with biochemical tools to investigate the localization and function of ABCB6 in pigment cells. We show that ABCB6 localizes to the membrane of early melanosomes and lysosomes of the human melanocytic cell line MNT-1. Depletion of ABCB6 by siRNA impaired PMEL amyloidogenesis in early melanosomes and induced aberrant accumulation of multilamellar aggregates in pigmented melanosomes. PMEL fibril formation and normal maturation of pigmented melanosomes could be restored by the overexpression of wild-type ABCB6 but not by variants containing an inactivating catalytic mutation (K629M) or the G579E DUH mutation. In line with the impairment of PMEL matrix formation in the absence of ABCB6, morphological analysis of the retinal pigment epithelium of *ABCB6* knockout mice revealed a significant decrease of melanosome numbers. Our study extends the localization of ABCB6 to melanosomes, suggesting a potential link between the function of ABCB6 and the etiology of DUH to amyloid formation in pigment cells.

PP-56: **Substrate specificity in the context of chlorophyll xenobiotics transport**

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There exist several levels of interactions of plant pigments chlorophylls (Chls) with animal organisms, however, the impact and interactions of chlorophyll-derived xenobiotics on animals and humans, related to the uptake of plant-based foods, was realized only recently. The strict dependence of the animal world on plants drove animals to evolve efficient defence mechanisms against tissue photosensitization due to the dietary Chls and photoactive products of their degradation. One of multidrug resistance proteins, BCRP (ABCG2), was found to play a key role in the protection against porphyrins and Chl derivatives of dietary origin. On the other hand, the ability of Chla derivatives for photosensitization, excellent light absorption properties and bioavailability make Chl derivatives a promising compounds used in photodynamic therapy of cancer, while the presence of a specific transporter may be a factor limiting the concentration and retention time of the drug in tissues, and thus its effectiveness.

In our earlier pharmacokinetic study, showing fate of various Chl-derived xenobiotics in mice organism, a large differences were seen between chlorophyllide a (Chlide) and its zinc analog (Zn-Pheide), despite only a slight difference in their structures. Based on a set of various modified Chl-derivatives, in a controlled manner differs in structure, we want to identify the determinants of substrate-transporter interactions.

To assess the substrate specificity of the ABCG2 protein in the context of Chl-derivatives, we used e.g.: (i) ATP-ase activity assay; (ii) flow cytometry assay; (iii) changes in the concentration of the compounds in cells with basal expression and overexpression of ABCG2; (iv) measurements of directional transport of the compound by polarized cells

PP-57: **ABCB1 nucleotide binding domain dimerization cycle**

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P-glycoprotein (a.k.a. MDR1 or ABCB1) is expressed at cellular barriers and causes resistance against chemotherapy if expressed in cancer cells. The pseudo-symmetric ABCB1 consists of two transmembrane domains (TMD) that bind and transport the substrate, and of two nucleotide binding domains (NBDs) that energize the transport by ATP binding and hydrolysis. It is accepted that dimerization is triggered by ATP binding, which switches the protein to the outward facing conformation and leads to cargo release to the extracellular side. Hydrolysis of ATP to ADP and inorganic phosphate eventually resets ABCB1 and induces the conformation of the inward conformation, which is of high affinity for substrate.

We study the energetics of NBD dimer formation and the role of nucleotides using biased molecular dynamics simulations of isolated NBDs. Potential of mean force profiles show that the apo configuration weakly favors the dimeric conformation, while a deep energy minimum (~ -42 kJ/mol) for the dimeric state is reached in the presence of ATP. Conserved motif interaction network analyses revealed that ATP stabilizes the NBD dimer by forming strong attractive interactions with both domains. These forces are multilayered and consist of electrostatic, hydrophobic and water mediated interactions between the nucleotide and the NBDs. ATP hydrolysis to ADP and HPO4 changes the potential and the forces. The free energy hypersurface reveals that the closed dimer became an unstable high energy state, therefore favoring the transition to an open conformation.

Our data show that ATP binding and its hydrolysis serve as sequential energy input, promoting respectively closure and the opening of the NBD dimer. The transport cycle of ABCB1 can be followed through the changes in the energy hypersurface.

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PP-58: **c-Met tyrosine kinase inhibitor tepotinib interacts with ABCB1 and ABCG2 drug efflux transporters as well as various CYP450 isoforms and attenuates pharmacokinetic multidrug resistance *in vitro***

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Tepotinib is a selective small-molecule inhibitor of c-Met receptor tyrosine kinase. Currently, this drug candidate shows promising results in several advanced-stage clinical trials for the therapy of solid tumors including lung cancer, one of the tumorous diseases, which therapy is often compromised by pharmacokinetic multidrug resistance (MDR) phenomenon. The aim of this study was to investigate the interactions of tepotinib with ABC (ATP-binding cassette) drug efflux transporters and cytochrome P450 (CYP450) isoforms as critical mechanisms controlling pharmacokinetic behaviour of drugs and playing also the role in MDR of cancer cells. First, we described the inhibitory activity of tepotinib toward human ABCB1, ABCG2 and ABCC1 using hoechst 33342 or calcein AM accumulation assays in MDCKII cells overexpressing respective ABC transporters. Drug combination studies (based on MTT assay) were employed to investigate the potential of tepotinib to overcome ABC transporter-mediated cytostatic MDR. Finally, using commercial Vivid CYP450 Screening kits, we evaluated tepotinib interactions with clinically relevant human CYP450 isoforms. Based on the results from accumulation studies, we described tepotinib as the potent inhibitor of ABCB1 and ABCG2 (IC50 of 3.782 and 16.08 µM, respectively), but not of ABCC1. Subsequent drug combination studies showed that tepotinib is able to effectively reverse daunorubicin and mitoxantrone resistance in MDCKII cells with ABCB1 and ABCG2 overexpression, respectively. Additionally, in experiments with Vivid CYP450 Screening kits, we demonstrated that tepotinib is a strong inhibitor of CYP3A4, CYP2C8, CYP2C9, CYP2C19, moderate inhibitor of CYP3A5 and does not affect activity of CYP1A2, CYP2D6 and CYP2B6 isoforms. In conclusion, our findings indicate that tepotinib interacts with ABCB1 and ABCG2 transporters and several isoforms of CYP450 metabolizing enzymes, and thereby shows high potential for clinically relevant drug-drug interactions. On the other hand, transporter-mediated interactions could be beneficially utilised for overcoming MDR. Future studies would be needed to help clarify the rationality of our conclusions *in vivo* and possibly enable more efficient and safe therapy to many oncological patients.

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PP-59: **Method development for the screening of yeast transporters using SSM-based electrophysiology**

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Production of compounds using microbial cell factories provides a sustainable alternative to their chemical synthesis. However, several issues such as feedback inhibition, product degradation and compound toxicity can make metabolic engineering of organisms for the production of compounds difficult. Using transporters in the engineering of organisms could help circumvent these problems.

Saccharomyces cerevisiae is a widely used organism for the production of chemicals and has approximately 315 putative transporters. Unfortunately, many of these transporters are currently not characterized and thus cannot be used for engineering of cell factories. To characterize transporters with unknown function, solid supported membrane (SSM)-based electrophysiology can be used, as the technique measures electrogenic reactions of transporters, e.g. transport of charged substrate[1]. The main advantage is that SSM-based electrophysiology can be scaled up to a high-throughput process for the characterization of transporters.

In order for us to characterize many transporters of *S. cerevisiae*, we have designed and set up a workflow for the expression and purification of transporters from yeast cells. We use the Yeast ORF Collection[2] to express transporters using an inducible promoter in yeast cells. Subsequently, we isolate crude membrane fractions from the cells, solubilize the proteins and purify them using a nickel-affinity column. To perform measurements, the proteins then have to be reconstituted into liposomes and finally measured using SSM-based electrophysiology on the SURFE^{2}R N1 machine using various solution exchange protocols.
Currently, we are optimizing and validating this methodology using yeast transporters with both known and unknown function, as well as testing whether the method can be applied for characterization of heterologous transporters. We will present the initial findings on the methodology design, validation and optimization for the characterization of transporters using yeast as an expression platform.

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PP-60: **An Allosteric Mechanism for Lipid inhibition of Glycine Transporter 2**

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The glycine transporter, GlyT2, is the target for a number of drugs that can alleviate neuropathic and inflammatory pain. We have developed a series of N-acyl amino acid analogues that inhibit GlyT2 and also show promise as leads for the development of a novel class of lipid-based analgesics. ~60 new N-acyl amino acids with varying head and acyl-tail groups were synthesised and found to be potent, selective GlyT2 inhibitors, 13 of which possess an IC_{50} < 100 nM, and provide analgesia in rat models of neuropathic pain. We have used site-directed mutagenesis combined with quantitative structure activity analysis to identify potential allosteric lipid binding sites and to understand communication between the lipid binding site and inhibition of transport. Mutations in four distinct regions of the protein influence the potency and extent of inhibition. Extracellular loop 4 undergoes considerable conformational changes in the transport cycle, and mutations in the loop (I545L, Y550L) disrupt inhibition by all lipids. A reverse mutation in EL4 of GlyT1 (L425I) introduces lipid sensitivity, which further reinforces the critical role of this domain in lipid binding and mechanism of inhibition. We reasoned that transmembrane domains that flank EL4 may also influence lipid inhibition. Mutations on the membrane-exposed external surface of TM8 (P561S, W563L) differentially reduce the potency of selected N-acyl amino acids and suggest potential lipid head group interactions with the transporter. Mutations of additional residues in TM8 (L569F) and TM5 (F428A, V432A) also reduce the potency of lipid inhibition in an acyl-tail specific manner, suggesting that this region binds the lipid tail. Finally, mutations in the middle of TM7 (eg. F515W) enhance the potency and also speed up the rate of recovery from inhibition, which suggest a role of TM7 in influencing accessibility of the lipids for their binding site. Coarse grain molecular dynamics simulations of lipids associating with GlyT2 suggest that the N-acyl amino acids enter the membrane and first associate with TM7 (near F515) and then intercalate between the transmembrane domains before reaching a stable binding site formed by EL4 and the extracellular halves of TM8 and TM5. A lipid-based inhibitor bound at this site is likely to restrict conformational changes in the transporter and thereby prevent the transport mechanism. We are currently using this information to further develop novel N-acyl amino acid GlyT2 inhibitors that may have therapeutic value in treating chronic pain.

PP-61: **A Third Binding Site on SERT for Novel Citalopram Analogs Identified by Photoaffinity Labeling**

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The neurotransmitter serotonin (5-hydroxytryptamine, 5HT) performs numerous crucial roles in the brain, including regulation of anxiety and mood, and disorders caused by imbalanced 5HT include Major Depressive Disorder (MDD) and Autism Spectrum Disorder (ASD). A critical regulator of synaptic 5HT levels is the serotonin transporter (SERT), which transports 5HT from the extracellular space into the neuron. SERT is the target for antidepressant drugs such as escitalopram (S-CIT, Celexa), and fluoxetine (Prozac), which bind to the transporter and inhibit its uptake activity, leading to elevated extracellular 5HT levels. These drugs are the most commonly prescribed medications for MDD, but their efficacy is moderate and many patients experience inadequate relief, indicating the need for better understanding of underlying mechanisms or development of novel therapeutics. Binding and inhibitory properties of antidepressant agents at SERT are complex, as many demonstrate high-affinity actions at the S1 orthosteric site, as well as allosteric modulation of S1 function mediated through low-affinity binding to a distinct S2 domain. To examine S-CIT binding mechanisms we developed two novel photoaffinity ligands, [(S)-3-(4-Azido-3-[^{125}I]iodophenyl)-N-((1-(3-(dimethylamino)-propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-yl)methyl)) propanamide], **([^{125}I]VK 03-83)**, and [(S)-1-(3-((4-Azido-3-[^{125}I]-iodophenethyl)(methyl)amino)propyl)-1-(4-fluorophenyl)-1,3-dihydro-isobenzofuran-5-carbonitrile)], **([^{125}I] VK 03-51),** that possess identical S-CIT pharmacophores but have cross-linking moieties appended to different positions of the citalopram core. Our findings show that both ligands bind to SERT, inhibit 5HT transport, and allosterically regulate S1 function, but display distinct pharmacological characteristics that indicate differential binding mechanisms. [^{125}I]VK 03-83 labeling of SERT is fully inhibited by 5HT and typical SERT inhibitors, with an S-CIT K_i of ~1 nM, consistent with binding to S1. In contrast, although [^{125}I]VK 03-51 labeling is fully displaced by the homologous ligand, it is inhibited only ~30% by S-CIT or other standard SERT inhibitors, and shows no displacement by 10 µM 5HT, indicative of binding to multiple pharmacologically distinct sites. The S-CIT-displaceable portion of [^{125}I]VK 03-51 labeling occurs with an S-CIT K_i of ~1 nM, consistent with this portion of binding occurring at S1. The remaining fraction of labeling that is not sensitive to ≥10 µM concentrations of S-CIT, other SERT inhibitors, or 5HT indicates mechanisms inconsistent with known properties of either S1 or S2, which should both be fully occupied in these conditions. These findings thus suggest that [^{125}I]VK 03-51 binds to SERT at a third site that has not been detected by reversible radioligand binding or co-crystallization approaches but is revealed by covalent cross-linking. This site may function in transport inhibition or allosteric regulation of S1 functions, and suggests a domain for exploration for transporter mechanisms or medication development.

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PP-62: **Label-Free Detection of Transporter Inhibitors' Efficacy In Living Cells**

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Background and purpose. Membrane transporters are transmembrane proteins that regulate the translocation of small molecules, inorganic ions or other proteins across biological membranes. Their function as gatekeepers of small molecules as well as their ubiquitous presence in the human body render them potential therapeutic targets, both for their activation and inhibition.

The current study aims to develop a label-free method for the assessment of transporter inhibitors by using the cellular impedance based xCELLigence equipment. This assay is taking advantage of the fact that endogenous substrates of membrane transporters are in the majority also ligands for membrane receptors (e.g., glutamate and dopamine are ligands for glutamate and dopamine receptors and they are transported by glutamate and dopamine transporters, respectively). As a result, inhibiting a transporter will often lead to an increased concentration of the endogenous substrate/ligand outside of the cell. This then may lead to an increase in receptor signaling which is monitored with the xCELLigence.

Experimental approach. To provide a proof-of-concept that a whole cell label-free assay can be applied to transporters, Equilibrative Nucleoside Transporter-1 (ENT1) was selected as a target. ENT1 is the most abundant nucleoside transport protein and one of its substrates is adenosine, which is also the endogenous ligand of adenosine receptors. The U-2 OS cell line was selected, which is a human osteosarcoma cell line that endogenously expresses both ENT1 and adenosine receptors.

Results. Three reference ENT1 inhibitors were tested in the new assay and as a result, changes in impedance were measured. In all cases the amount of adenosine outside the cell membrane appeared increased when ENT1 was inhibited, as changes in impedance were more profound compared to a control with no ENT1 inhibition. This observation triggered further experiments that allowed a quantitative assessment of the inhibitors' potency.

Conclusions. Our research is aimed at the development of an innovative label-free assay that can potentially be used for a multitude of membrane transporters as an *in vitro* model system for the evaluation of transporter inhibitors. The new format is not limited by expensive and specific tags and labels, such as radioactivity. More generally, this approach could inspire future drug discovery in the field of membrane transporters.

PP-63: **Serotonin in the Medial Prefrontal Cortex: How Transport is Altered in Autism Spectrum Disorder Models**

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Autism Spectrum Disorder (ASD) is the fastest growing developmental disability and currently affects 1 in 68 children in the US. Despite the increased prevalence, a single etiology has not been identified, but many experts agree that serotonin is a reproducable biomarker as roughly 1/3rd of individuals with ASD having high blood serotonin levels. A clear correlation between serotonin levels in the periphery and the central nervous system does not exist since serotonin cannot cross the blood brain barrier. To accurately asses serotonin neurotransmission, we use fast-scan cyclic voltammetry which allows us to make *in vivo* serotonin measurements in real time in both genetic and environmental risk factor exposure models. The genetic models used here, SHANK3 and SERT-Ala56, have previously been verified to have behavioral phenotypes associated with ASD mouse models. Likewise, to verify our model of perinatal lead exposure, we implement behavioral testing to evaluate the ASD behavioral phenotype. Preliminary results suggest the genetic models exhibit alterations in the transporters that remove serotonin from the extracellular space. The lead exposure model has demonstrated alterations in behavior as well as disruptions in serotonin neurotransmission.

PP-64: A triple-fluorescence cell line system for the high throughput probing of substrates for ABCB1, ABCG2 and OATP2B1

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Plasma membrane proteins play an important role in the cellular uptake and efflux of nutrients and xenobiotics. By regulating absorption, distribution, metabolism and excretion (ADME), drug transporters can confer sensitivity or resistance to anticancer drugs. Multidrug resistance phenotype in tumors is associated with the overexpression of ABC efflux transporters, termed multidrug resistance (MDR) proteins. One of the major group of SLC uptake transporters involved in ADME processes belong to the OATP (Organic Anion Transporting Polypeptides, SLCO) family. Here we introduce a HTS amenable cytotoxicity assay based on the co-culturing of fluorescent parental and transporter-expressing cells. eGFP expressing parental A431 cells were cocultured with either A431 cells stably expressing mCherry (mCh) and ABCB1/P-glycoprotein (Pgp) or mOrange (mOr) and ABCG2. In addition, we transfected the human sarcoma cell line Mes-Sa and its multidrug-resistant counterparts (Mes-Sa/Dx5 and Mes-Sa-B1) with mCh, eGFP and mOr, respectively. To study the relevance of uptake transporters we engineered A431 cells overexpressing OATP-2B1 and mCherry.

We used the established co-culture model system of the fluorescent protein expressing cell lines to characterize the ability of the transporter proteins to modulate the cytotoxic activity of 101 clinically used chemotherapeutics compiled in the Developmental Therapeutics Program's (DTP) Oncology Set IV. *In addition* to identifying known substrates of ABCB1 and ABCG2, the fluorescence-based cytotoxicity assays identified 13 putative OATP2B1 substrates whose toxicity was increased in OATP2B1 expressing cells. Interaction of these compounds with OATP2B1 was verified in dedicated transport assays using novel cell-impermeant fluorescent substrates[1]. Using decision tree based classification strategies, we identified structural determinants associated with the increased toxicity of the 13 compounds.

Our results highlight the potential of the fluorescence-based HT screening system for identifying transporter substrates.

[1] Patik et al: Identification of novel cell-impermeant fluorescent substrates for testing the function and drug interaction of Organic Anion-Transporting Polypeptides, OATP1B1/1B3 and 2B1. Sci Rep 8:2630; 2018

PP-65: **Dopamine transporter function is regulated by protein phosphatase PP1/2A through a novel phosphorylation site**

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Several protein kinases including protein kinase C, Ca^{2+}/calmodulin-dependent protein kinase II, and extracellular regulated kinase play key roles in the regulation of dopamine transporter (DAT) functions such as endocytosis, dopamine uptake and reverse transport, with phosphorylation sites for these kinases linked to distinct regions of the N-terminus. Protein phosphatases (PPs) also regulate DAT activity but their specific residues associated with their activities have not yet been elucidated. In this study, we identified DAT-associated PP1 and PP2A in the mouse brain and heterologous cell systems by co-immunoprecipitation followed by mass spectrometry (MS) and immunoblotting. By applying MS in conjunction with a metabolic labelling method, we further defined the PP1/2A-sensitive phosphorylation site at Thr48 in human DAT, a residue that has not been previously identified. Site-directed mutation at Thr48 (T48A) prevented phosphorylation and led to enhanced dopamine transport kinetics, supporting the functional role of dephosphorylation of this residue. In addition, increased palmitoylation in the dephosphomimetic T48A DAT mutant reinforced previously reported reciprocal DAT regulation between de/phosphorylation and palmitoylation.

PP-66: **Stochastic modeling of glutamate transporter functioning**

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L-glutamate is the major mammalian excitatory amino acid but its excessive stimulation can result in excitotoxicity (glutamate-related cell injury or death).The precise control of glutamatergic synaptic transmission is performed by Excitatory amino acid transporters (EAATs 1-5) which operate as secondary active transporters and ion channels at the same time. An impaired EAATs functioning may contribute to a large number of severe neurodegenerative disorders. Therefore, unambiguous understanding of the glutamate transporter functional mechanism and its kinetic properties is of the great importance.

EAATs are trimeric proteins. Each protomer is an independently functioning subunit which accomplishes a co-transport of 1 glutamate with 3 sodium ions and 1 proton into the cytosol, and counter-transport of 1 potassium ion into the external solution. Protein undergoes several conformational changes during its whole transport cycle. The conformational changings related to association events leads to additional protein transformations which possess anion channel activity. One of unsettled question in EAATs functioning is the sequence of elementary steps of substrate and co-transported ions associations on the extracellular part of the membrane and consequently its dissociation on the intracellular part.

Virtual simulator was created to examine the kinetic properties of a single glutamate transporter. At the core of it is stochastic modeling algorithm based on probability coefficients of each elementary step of the protein transport cycle. The structural properties of the protein are implicit in a probabilities value, which generally derived from equilibrium constants of each elemental reaction (step) or differences of free energies of the substrates binding. The timescale parameters of each step could be settled independently. Such an approach provides an opportunity to change the sequence of elementary events and so to take into account different schemes of protein transport cycle. Besides, virtual experiments could be performed at different ambient conditions at a rather short time.

Along with permutation with repetitions there are 400 variants of scheme for sequence of substrate association and dissociation elementary steps in glutamate transporters. All of them were examined. The dependences describing their turnaround kinetic properties exhibit qualitative characterization and the time course of the steps is to be validated. Under physiological conditions transport kinetics of EAATs doesn’t depend on the dissociation steps order. The association steps order has insufficient impact on maximum rate achieved by the system but leads to changing in enzyme affinity for the substrate. On the base of EAATs activity computer modeling it was shown that the most optimal sequence of elemental events is implemented in the scheme wherein glutamate molecule associates to the protonated form of the transporter after the first sodium ion binding.

PP-67: **Profiling selectivity of hepatic Organic Anion Transporting Polypeptides by combining *in silico* and *in vitro* methods**

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The hepatocellular Organic Anion Transporting Polypeptides OATP1B1, OATP1B3, and OATP2B1 are important for drug elimination and proper liver function. Thus, predicting potential interactions with liver OATPs is of utmost importance. Still, knowledge about the molecular basis for hepatocellular OATP-ligand interactions and their selectivity is very restricted mainly due to the lack of a high resolution crystal structure of any OATP.

Up to now, only very few computer-generated structural models are available for hepatocellular OATPs and no systematic large-scale docking study has been performed so far. In addition, a comparative study for characterization of the structure-function relationship of all three human hepatocellular OATPs is missing.

We are following an interdisciplinary research approach, combining ligand- and structure-based *in silico* methods with biochemical *in vitro* studies. To date, we have compiled comprehensive high-quality sets of substrates and inhibitors for all three hepatic OATPs by integrating data from five different open data sources with a semi-automatic workflow. The data mining efforts led to a gain in compound and chemical space, as well as to an increase in the confidence of the activity annotations [(non-)substrates, (non)-inhibitors]. Subsequently, the data was analyzed with respect to enriched scaffolds as well as chemical features potentially being relevant for selectivity towards one or two of the transporters over the other(s).

Next, we will use the curated data sets for performing docking studies into homology models (by utilizing template structures from the Major Facilitator Superfamily which are possessing highest secondary structural similarity). Experimental verification of the binding mode hypotheses will be performed by mutational studies and novel potential ligands of hepatocellular OATPs will be predicted *in silico* by virtual screening of large databases. *In vitro* testing will be performed in a recently developed fluorescence-based assay using A431 cell lines engineered to overexpress human OATPs, 1B1, 1B3 and 2B1.[1]

Insights about new drug-OATP interactions, will further aid in preventing clinically important drug-drug interactions between co-administered drugs. In addition, the identification of novel selective tool compounds for hepatic OATPs will aid in diagnosis and functional characterisation of these relatively uncharted uptake-transporters.

[1] *Patik et al., Sci Rep 2018, 8:2630.*

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PP-68: **Two binding sites in the dopamine transporter revealed by force spectroscopy**

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The molecular structure of the drosophila dopamine transporter (dDAT) constructed from X-ray crystallography revealed that the binding site for substrates (e.g. dopamine) and for inhibitors (e.g. cocaine) is located in the center of the transporter. However, computational docking and molecular dynamic simulation studies suggested that there might be a second binding site in the extracellular vestibule of DAT. Binding experiments using dopamine dimers with various linker molecules and spacer lengths supported this notion. X-ray structures of closely related serotonin transporter (SERT) and leucine transporter (LeuT) showed a second binding site in the extracellular vestibule. In the present study, we utilized atomic force microscopy (AFM) to directly measure the interaction forces between DAT and various ligands. Force measurements between DAT and the cocaine analogue MFZ2-12 revealed that two populations with different binding strength existed and that the binding activity was increased in the presence of 10 µM Zn^{2+}. These findings are in accordance with the proposed stabilization of the outward facing conformation by Zn^{2+}. The absence of Na^{+}, Zn^{2+}, or introduction of the mutation S422A or V152I dramatically reduced the population displaying strong interaction forces, indicating that it originated from binding to the central S1 binding site. On the other hand, protonating the histidine residue H477 significantly reduced the population of weaker interaction forces, suggesting that it had originated from binding to the extracellular vestibule. Two populations of unbinding forces were also observed with AFM tips conjugated with a novel dopamine analogue or dopamine itself. Substitution of Na^{+} in the buffer with K^{+} or $NMDG^{+}$, or introduction of the mutation V152I disrupted the population of stronger binding, whereas acetylation of the lysine residues K92 and K384 in the extracellular vestibule diminished the population of weaker binding. Thus, single molecule force spectroscopy, which allows for the extraction of dynamic information of transmembrane transporters in the native cellular membrane under physiological conditions, provides clear physical evidence of two distinct binding sites in DAT.

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ITMTS International Transmembrane Transporter Society

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