Closing Conference of the Doctoral Program Ion Channels and Transporters as Molecular Drug Targets

Funded by the Austrian Science Fund (FWF)

Abstracts

CONTENTS:

Session Talks – Session 3 (26.09., 09:15 – 10:50)

Session Talks – Session 4 (26.09., 13:15 – 14:50)

5 – Abstracts of the Poster Session (Sept 25, from 15:25)

Plenary Session 1, Monday, Sept 25, 09:15; Session (Host: Marco Niello)

Lynette C. Daws, PhD
University of Texas Health Science Center at San Antonio
Departments of Cellular & Integrative Physiology and Pharmacology

More than one way to transport monoamines: Uncovering novel molecular targets for therapeutics to treat psychiatric and substance use disorders

Monoamines, including dopamine and serotonin, are essential modulators of a variety of physiological and neural processes. Imbalances in monoamine signaling are strongly linked to a number of pathologies including substance use and psychiatric disorders. The strength and duration of monoamine signaling is tightly controlled by so-called "uptake-1" transporters, including the dopamine and serotonin transporters (DAT and SERT). These are high-affinity transporters for their substrates but have a low capacity to do so when extracellular concentrations of dopamine and serotonin are high. Not surprisingly, these transporters are the targets for numerous therapeutic drugs to treat disorders where imbalances in dopamine and serotonin homeostasis are considered causative. However, these therapeutics largely provide suboptimal symptom relief for the majority of patients. Why? Until the late 1990s it was generally accepted that there is only one transporter for each neurotransmitter, DAT for dopamine, SERT for serotonin, and so on. The idea of transporter "promiscuity" was not widely accepted. This presentation will begin with our initial finding in 1998 that the norepinephrine transporter is a very capable transporter of serotonin, and how this led us to uncover organic cation transporter 3 (OCT3) as an important mechanism for serotonin and dopamine transport, as well as a site of action for amphetamine and alcohol. Our findings point to OCT3 as a promising therapeutic target for psychiatric and substance use disorders. We are currently working with medicinal chemists to develop compounds selective for OCT3, which can be used in the clinic.

Plenary Session 1, Monday, Sept 25, 10:00 (Host: Marco Niello)

Name / affiliation: Stefanie Kickinger, University of Copenhagen

Title: Development of novel selective tool compounds for the nutrient amino acid transporters (SLC6A15-20)

Abstract

The six nutrient amino acid transporters (NAATs, SLC6A15-20) are structurally and functionally highly related to the neurotransmitter transporters. Although several of these transporters are potentially implicated in metabolic and neurological diseases, they are remarkably understudied.[1] Recently, 3D structures of SLC6A19 and SLC6A20 were resolved in complex with angiotensin converting enzyme 2 (ACE2) and the receptor-binding domain of SARS-CoV-2.[2,3] SLC6A20 was further shown to be increasingly expressed in patients suffering from severe COVID-19 caused by SARS-CoV-2.[4] This suggests that the NAATs, particularly SLC6A18-A20 which are co-expressed with ACE2, may play a role in the internalization of the virus and thus represent a novel therapeutic strategy. There are currently no potent tool compounds available that selectively inhibit or modulate NAAT functions. Hence, the general role of the NAATs in pathophysiology and their potential as new drug targets remain elusive. In this work, we developed radioligand uptake assays for screening an in-house drug-like compound library for SLC6A17, SLC6A18 and SLC6A20 in mammalian cells and Xenopus laevis oocytes. Additionally, a virtual screening workflow of Enamine libraries (HTS, Advanced, Premium) and DrugBank was created. Here we present the results of our screening efforts that bring us one step closer to the development of novel selective and active tool compounds.

1. Bröer, S. (2018). SLAS DISCOVERY: Advancing Life Sciences R&D 23, 303–320. 10.1177/2472555218755629 2. Yan, R. et al. (2020). Science 367, 1444–1448. 10.1126/science.abb2762. 3. Shen, Y. et al. (2022). Cell Discov 8, 123. 10.1038/s41421-022-00488-x. 4. Ellinghaus, D. et al. (2020). N Engl J Med. 10.1056/NEJMoa2020283.

Plenary Session 1, Monday, Sept 25, 10:25 (Co-Host 1: Nina Kastner)

Name / affiliation: Thomas Steinkellner, Medical University of Vienna

Title: Selective vulnerability of dopamine neurons in Parkinson's disease

Abstract

Though many neuronal populations are affected in Parkinson's disease (PD), its cardinal motor symptoms are a consequence of dopamine (DA) neuron loss in the substantia nigra (SNc). The precise mechanisms underlying DA neuron vulnerability remain unclear, but include oxidative stress, and aggregation of alpha-synuclein. More recently, a glutamate driven process has been implicated in disease progression, and there is now proof that DA neurons express the vesicular glutamate transporter VGLUT2 and co-release glutamate. Further, there is evidence for a presynaptic role of VGLUT2, whereby VGLUT2 can increase the driving force for loading DA into synaptic vesicles, which may enable tuning of DA release in response to activity changes. We recently discovered that the majority of SNc DA neurons transiently express VGLUT2 in development, but most shut down expression in the adult. Interestingly, VGLUT2 can re-emerge in response to insult. Reemergent VGLUT2 may provide a beneficial compensatory adaptation and contribute to the native resistance of ventral tegmental area DA neurons that express more VGLUT2. Consistent with this, we find that DA neurons are more sensitive to toxin-induced cell death in mice that lack VGLUT2 in DA neurons; and DA neurons expressing VGLUT2 are more resilient to neuronal injury in animals and in human PD. On the other hand, we find that ectopic expression of VGLUT2 causes profound toxicity to SNc DA but not other neuronal populations.

We propose that VGLUT2 confers protection to DA neurons with low VGLUT2 levels in adult DA neurons and little co-release restricted to synaptic release sites. In response to increased metabolic demand, neural activity, aging or injury, VGLUT2 transcription temporarily increases to sustain DA transmission or to sequester toxic substrates to vesicles. However, severe or sustained injury leads to prolonged or high-level expression of VGLUT2 in DA neurons and vulnerable populations cannot cope with the consequences.

Authors:

Thomas Steinkellner, Medical University of Vienna

Thomas Hnasko, University of California, San Diego

.

Name / affiliation: Kusumika Saha, Brigham's Women Hospital

Title: Pharmacological chaperone-rescued cystic fibrosis CFTR-F508del mutant overcomes PRAF2-gated access to endoplasmic reticulum exit sites

Abstract

Kusumika Saha[1 2], Benoit Chevalier[3], Stéphane Doly[1 4], Nesrine Baatallah [3], Thomas Guilbert[1], Iwona Pranke[3], Mark G H Scott[1], Hervé Enslen[1], Chiara Guerrera[5], Cérina Chuon [5,] Aleksander Edelman[3], Isabelle Sermet-Gaudelus[3 6], Alexandre Hinzpeter [#3], Stefano Marullo[# 7]

Affiliations

[1]Université Paris Cité, Institut Cochin, INSERM U1016, CNRS UMR 8104, 75014, Paris, France.
[2]Division of Cardiovascular Medicine, Brigham and Women's Hospital, Boston, MA, 02115, USA.
[3]Institut-Necker-Enfants-Malades, Université Paris Cité, INSERM U1151, CNRS UMR 8253, 75015, Paris, France.
[4]Université Clermont Auvergne, INSERM U1107, NEURO-DOL, 63000, Clermont-Ferrand, France.
[5]Proteomic Facility, Institut-Necker-Enfants-Malades, Université Paris Cité, 75015, Paris, France.
[6]Centre de Référence Maladies Rares Mucoviscidose et Maladies de CFTR, Hôpital Necker Enfants Malades, European Reference National (ERN) Lung Center, Paris, France.
[7]Université Paris Cité, Institut Cochin, INSERM U1016, CNRS UMR 8104, 75014, Paris, France.
stefano.marullo@inserm.fr.
#Contributed equally.

Partially characterized retention mechanisms involving arginine-based motifs, inhibit Cystic Fibrosis (CF) Transmembrane Conductance Regulator (CFTR) exit from the endoplamic reticulum (ER). The mutagenesis of these RXR motifs partly rescued the transport to the cell surface of the CFTR-F508del mutant found in CF patients.

The GB1 subunit of the metabotropic $GABA_B$-receptor is fully retained in the ER in the absence of the GB2 subunit. GB1 retention depends on a di-leucin and a RXR motif, which are masked when GB1 hetero-dimerizes with GB2, allowing the release of a competent $GABA_B$-receptor heterodimer to the cell surface. PRAF2, an ER gatekeeper, which recognizes these motifs, was shown to play a major role in GB1 subunit retention.

We found that PRAF2 can also interact on a stoichiometric basis with di-leucin and RXR motifs present in the NBD1 domain of both wild type and mutant CFTR, preventing their access to ER exit sites. Overexpression of PRAF2 inhibits cell surface expression of wild type CFTR in a concentration-dependent manner. Because of its lower abundance, compared to wild type CFTR, CFTR-F508del recruitment into COPII vesicles is suppressed by endogenous ER-resident PRAF2. Interestingly, some of the new pharmacological chaperones that efficiently rescue CFTR-F508del loss of function in CF patients, target CFTR-F508del retention by PRAF2 operating with various mechanisms.

These findings open new therapeutic perspectives for rare diseases caused by the impaired cell surface trafficking of misfolded transporters or receptors.

Name / affiliation: Felix P. Mayer, University of Copenhagen

Title: Kappa Opioid Receptor Antagonism Rescues Genetic Perturbation of Dopamine Homeostasis: Molecular, Physiological and Behavioral Consequences

Abstract

Felix P Mayer, Adele Stewart, Durairaj Ragu Varman, Amy E Moritz, James D Foster, Anthony W Owens, Lorena B Areal, Raajaram Gowrishankar, Michelle Velez, Kyria Wickham, Hannah Phelps, Rania Katamish, Maximilian Rabil, Lankupalle D Jayanthi, Roxanne A Vaughan, Lynette C Daws, Randy D Blakely, Sammanda Ramamoorthy

Aberrant dopamine (DA) signaling is implicated in schizophrenia, bipolar disorder (BPD), autism spectrum disorder (ASD), substance use disorder, and attention-deficit/hyperactivity disorder (ADHD). Treatment of these disorders remains inadequate. We established that the human DA transporter (DAT) coding variant (DAT Val559), identified in individuals with ADHD, ASD, or BPD, exhibits anomalous DA efflux (ADE) that is blocked by therapeutic amphetamines and methylphenidate. As the latter agents have high abuse liability, we exploited DAT Val559 knock-in mice to identify non-addictive agents that can normalize DAT Val559 functional and behavioral effects *ex vivo* and *in vivo*. Kappa opioid receptors (KORs) are expressed by DA neurons and modulate DA release and clearance, suggesting that targeting KORs might offset the effects of DAT Val559. We establish that enhanced DAT Thr53 phosphorylation and increased DAT surface trafficking associated with DAT Val559 expression are mimicked by KOR agonism of wildtype preparations and rescued by KOR antagonism of DAT Val559 *ex vivo* preparations. Importantly, KOR antagonism also corrected *in vivo* DA release and sex-dependent behavioral abnormalities. Given their low abuse liability, our studies with a construct valid model of human DA associated disorders reinforce considerations of KOR antagonism as a pharmacological strategy to treat DA associated brain disorders.

Plenary Session 1, Monday, Sept 25, 12:00 (Co-Host 2: Ralph Gradisch)

Name / affiliation: Eva Hellsberg, National Institutes of Health

Title: The molecular puzzle of the human serotonin transporter: where a proton and a potassium ion fit in the picture

Abstract

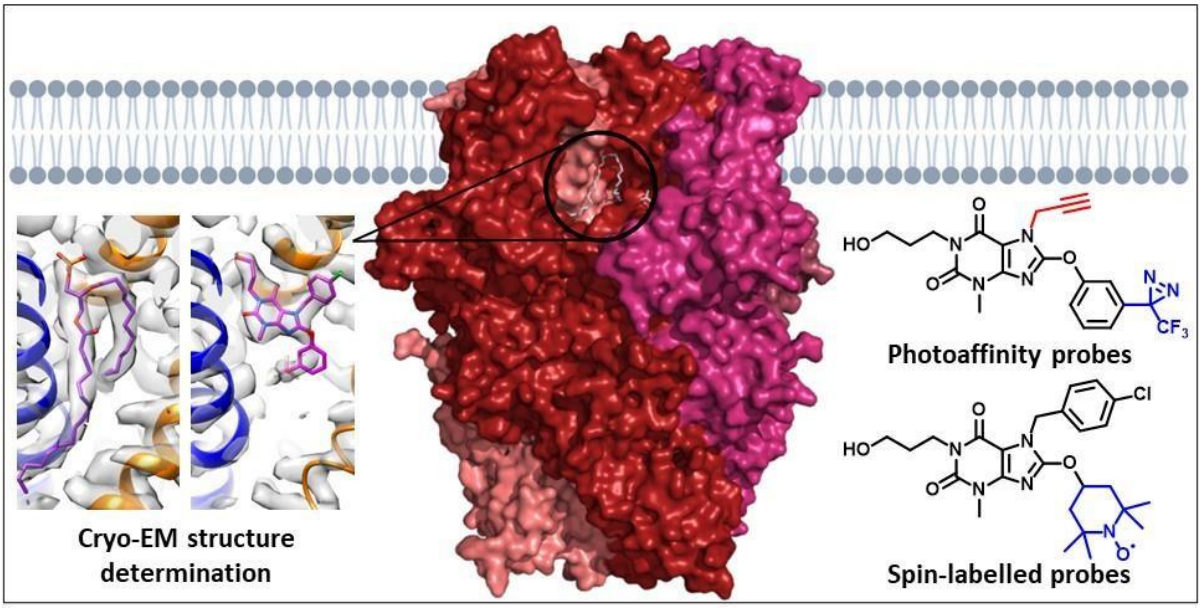
Eva Hellsberg[1], Danila Boytsov[2], Walter Sandtner[2], Lucy R. Forrest[1]
[1]Computational Structural Biology Unit, NINDS NIH, Bethesda, MD, USA2
[2]Department of Pharmacology, Medical University of Vienna, Austria

According to a recent WHO estimation, about 5 percent of adults globally suffer from major depression disorder. The underlying pathophysiology is not understood completely. However, insufficient monoamine neurotransmitter activity, especially of serotonergic nature, does evidently play a major role. Serotonin activity is regulated by the serotonin transporter SERT, a sodium-dependent membrane protein belonging to the solute carrier 6 family. SERT terminates the serotonin signal in the synaptic cleft by reuptake into presynaptic neurons, and is the drug target of all selective serotonin reuptake inhibitors, the most widely prescribed antidepressant drugs. Despite decades of extensive research efforts, many fundamental questions around serotonin functionality and depression treatment still remain unanswered. One critical knowledge gap is the exact serotonin transport stoichiometry under physiological conditions, a key element to understanding the uptake mechanism. Several experimental 3D structure series revealed or confirmed important structural features of the transporter. Nonetheless, the binding sites for a potassium ion and a proton, which are part of the physiological transport cycle, have not yet been identified. To this end, we have applied molecular dynamics simulations at atomistic detail and systematically tested all possible proton/ion combinations. We developed 16 different protein-membrane simulation systems comprising about 150000 atoms each, and calculated more than 30 microseconds simulation time in total. The results from these extensive sampling efforts indeed predict the locations of the potassium and proton binding sites. Moreover, examination of the interaction networks suggest the molecular origins of selectivity at each potential binding site. All our computational predictions are verified by biochemical fluorescence uptake experiments and electrophysiological measurements using the whole-cell patch-clamp technique, provided by our collaborators. Resulting from our interdisciplinary approach, we present a promising model explaining the serotonin transport stoichiometry on the molecular level with all its components for the first time. Beyond the gains in basic scientific understanding, the knowledge obtained paves the way for designing new mechanism-based transporter ligands with the potential to improve depression therapy and more.

Plenary Session 2, Monday, Sept 25, 13:15 (Host: Viktor Savic)

Robin S. Bon; r.bon@leeds.ac.uk
Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds LS29JT, UK and Astbury Centre for Structural Molecular Biology

Understanding TRPC1/4/5 channels through chemical biology andstructural pharmacology



TRPC1/4/5 proteins form homo- or heterotetrameric, non-selective cation channels permeable by Na^+ and Ca^{2+}. These channels are receiving increasing attention from both academia and industry as potential drug targets for the treatment of, for example, cardiovascular diseases and disorders of the central nervous system. In recent years, several highly potent and selective TRPC1/4/5 modulators have been reported, some of which have formed the basis for the development of clinical candidates. However, the molecular mode-of-action of these compounds was poorly understood.

We have used an integrated chemical/structural biology approach to study TRPC1/4/5 channel pharmacology, including: 1) detailed functional profiling of TRPC1/4/5 modulators; 2) determination of structure-activity relationships; 3) determination of high-resolution (2.4-3.0 Å) cryo-EM structures; 4) development of photoaffinity probes and covalent inhibitors; 5) development of channel variants (point mutants, chimaeras, reactive tags); development of spin-labelled probes for EPR-based studies. Through these combined efforts, we have gained detailed insight into the modulation of TRPC1/4/5 channels by small molecules and endogenous factors such as lipids and metal ions, allowing structure-guided design of new chemical probes.

Acknowledgements: This work is the result of the efforts of a large interdisciplinary team ofstudents, postdocs, facility staff and collaborators, all of whom will be duly acknowledged.

References: Bon RS et al. *Ann. Rev. Pharmacol. Toxicol.* **2022**, *62*, 427; Bauer CC et al. *RSC Chem. Biol.* **2020**, *1*, 436; Wright DJ et al. *Commun. Biol.* **2020**, *3*, 704

Name / affiliation: Konstantina Bampali, University of Copenhagen

Title: Antipsychotics and antidepressants can inhibit α5-containing $GABA_A$ receptorsby two distinct mechanisms

Abstract:

Konstantina Bampali[1]| Filip Koniuszewski[1]| Luca L. Silva[1]| Sabah Rehman[2]| Florian D. Vogel[1]| ThomasSeidel[3]| Petra Scholze[1]| Florian Zirpel[1]| Arthur Garon[3]| Thierry Langer[3]| Matthäus Willeit[4]| Margot Ernst[11]Department of Pathobiology of the Nervous System, Center for Brain Research, Medical University Vienna, Vienna, Austria
[2]Department of Molecular Neurosciences, Center for Brain Research, Medical University of Vienna, Vienna, Austria
[3]Department of Pharmaceutical Sciences, Division of Pharmaceutical Chemistry, University of Vienna,Vienna, Austria
[4]Department of Psychiatry and Psychotherapy, Medical University of Vienna, Vienna, Austria

Many psychotherapeutic drugs, including clozapine, display polypharmacology and act on $GABA_A$ receptors. Patients with schizophrenia show alterations in function, structure and molecular composition of the hippocampus, and a recent study demonstrated aberrant levels of hippocampal α5 subunit-containing $GABA_A$ receptors. The purpose of this study was to investigate the effects of tricyclic compounds on α5 subunit-containing receptor subtypes. Functional studies of effects by seven antipsychotic and antidepressant medications were performed in several $GABA_A$ receptor subtypes by two-electrode voltage-clamp electrophysiology using *Xenopus laevis* oocytes. Computational structural analysis was employed to design mutated constructs of the α5 subunit, probing a novel binding site and radioligand displacementdata complemented the functional and mutational findings. The antipsychotic drugs clozapine and chlorpromazine exerted functional inhibition on multiple $GABA_A$ receptor subtypes, including those containing α5-subunits. Based on a chlorpromazine binding site observed in a GABA-gated bacterial homologue, we identified a novel site in α5 $GABA_A$ receptor subunits and demonstrate differential usageof this and the orthosteric sites by these ligands. Despite high molecular and functional similarities amongthe tested ligands, they reduce GABA currents by differential usage of allosteric and orthosteric sites. Thechlorpromazine site described in this study is a new potential target for optimizing antipsychotic medications with beneficial polypharmacology. Further studies in defined subtypes are needed to substantiate mechanistic links between the therapeutic effects of clozapine and its action on certain $GABA_A$ receptor subtypes.

Plenary Session 2, Monday, Sept 25, 14:25 (Host: Viktor Savic)

Name/affiliation: Lukas Rycek, Charles University Prague

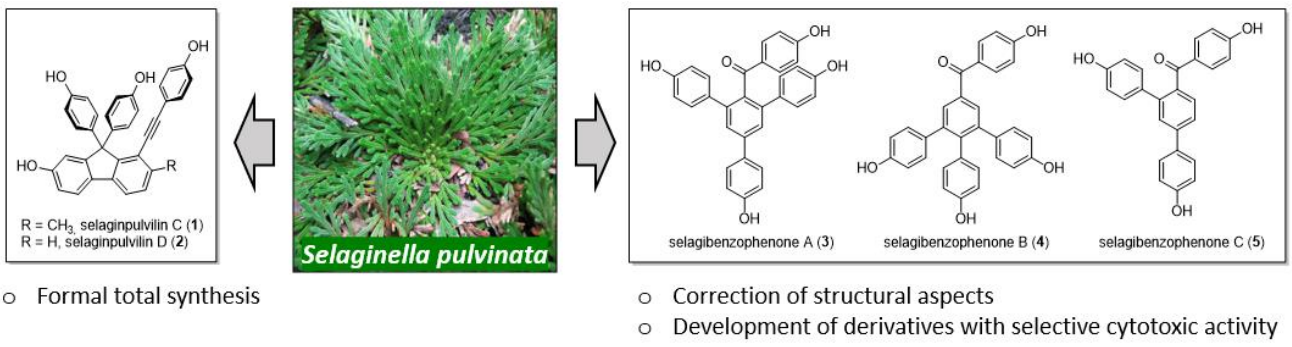
Title: *Selaginella* Natural Products as a Platform for Evaluation of New Synthetic Methods, Structural Aspects Elucidation and Drug Discovery

Lukas Rycek, Ringaile Lapinskaite, Miguel Mateus, Dominik Kunák, Štefan Malatinec

Department of Organic Chemistry Faculty of Science, Charles University, Czech Republic

Natural products have been interfering with human activities since ancient times. Their foremost role, without doubt, was their use in folklore medicines. Nowadays, they are still in a center of the attention of scientists from different fields. For synthetic organic chemists, the total synthesis of natural products is a privileged discipline. Perhaps there is no better way to evaluate the relevance of a new synthetic method, than its application in total synthesis. Moreover, despite the advances in analytical methods, errors in the structure elucidation of natural products still occur in literature. Total synthesis remains an irreplaceable tool for solving structural discrepancies. Last but not least, natural products play a substantial role in drug development.[1]

In this contribution, the aspects discussed above will be exemplified in the context of our research focused on the natural products from the plants of the genus *Selaginella*. First, the formal total synthesis of selaginpulvilins C (**1**) and D (**2**) using catalytic [2+2+2]-cyclotrimerization as a key step will be discussed.[2] Furthermore, the synthesis of selagibenzophenones A-C (**3-5**) will be described, and light will be shed on the structural discrepancies present in the literature.[3,4] Last but not least, the development of derivatives of these compounds with a selective cytotoxic profile will



be discussed as well.[5]

Figure 1. *Selaginella pulvinata* and natural products selaginpulvilins C (**1**), D (**2**), and selagibenzophenones A-C (**3-5**).

References:

(1) L. Rycek, *Chemické Listy* **2022**, *116*, 204-2014. (2) L. Rycek, M. Mateus, N. Beytrlerová, M. Kotora *Org. Lett.* **2021**, *23*, 4511. (3) R. Lapinskaite, Š. Malatinec, M. Mateus, L. Rycek *Catalysts* **2021**, *11*, 708. (4) D. Kunák, M. Mateus, L. Rycek *Eur. J. Org. Chem.* **2022**, e202200014. (5) R. Lapinskaite, *et al. Chemistry Select* **2023**,*8*, e20220481.

Plenary Session 2, Monday, Sept 25, 14:25 (Co-Host: Florian Vogel)

Name / affiliation: Kumaresan Jayaraman, Laurus Bio Bangalore

Title: Enzyme engineering to resist feedback inhibition in 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase

Abstract

Kumaresan Jayaraman[1], Natalia Trachtmann[2], Georg A. Sprenger[2], Holger Gohlke[1,3]

1 Institut für Pharmazeutische und Medizinische Chemie, Heinrich-Heine-Universität Düsseldorf, 40225 Düsseldorf, Germany
2 Institute of Microbiology, University of Stuttgart, Allmandring 31, 70569 Stuttgart, Germany
3 Jülich Supercomputing Centre (JSC), Institute of Biological Information Processing (IBI-7: Structural Biochemistry), John von Neumann Institute for Computing (NIC), & Institute of Bio- and Geosciences (IBG-4: Bioinformatics), Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

The shikimate pathway is responsible for producing aromatic amino acids (AAAs) in prokaryotes, fungi, and plants. This pathway is heavily utilized in the industrial synthesis of bioactive compounds. The enzyme 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DAHPS) controls carbon flow into this pathway. Feedback inhibition from downstream AAAs, such as phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp), regulates DAHPS. In Corynebacterium glutamicum, which is widely used for amino acid production, there are two isoenzymes of DAHPS: AroF (Tyr sensitive) and AroG (Phe and Tyr sensitive). This study aimed to introduce feedback resistance against Tyr in the class I DAHPS AroF (AroFcg). The approach used a consensus method, incorporating structural modeling, sequence and structural comparisons, knowledge of feedback-resistant variants in E. coli homologs, and computed folding free energy changes. Two types of variants were predicted, one where substitutions destabilize the inhibitor binding site and the other where substitutions directly interfere with inhibitor binding. Eight AroFcg variants were produced, purified, and assessed in enzyme activity assays in the presence or absence of Tyr. Two of these variants (E154N and P155L) had over 80% and over 50% residual activity, respectively, at 5 mM Tyr. Additionally, these variants exhibited over 50% specific activity of the wild-type AroFcg in the absence of Tyr. Further evaluation of two and four additional variants at positions 154 and 155 resulted in the discovery of E154S, which was completely resistant to 5 mM Tyr, and P155I, which behaved similarly to P155L. These feedback-resistant variants are unlikely to evolve by point mutations from the parental gene and would be missed by classical strain engineering.

Plenary Session 3, Tuesday, Sept 26, 09:15 (Host: Theres Friesacher)

Marina A. Kasimova
Senior Scientist at Novo Nordisk Denmark

From understanding principles of ion channel function to design of protein modulators

Understanding principles of protein function is crucial for design of protein modulators. Throughout my academic career, I've been studying different types of ion channels, from those responding to voltage and involved in action potential to those regulated by temperature and inflammation factors and involved in pain sensation. When I changed my career path to work at Novo Nordisk, knowledge about protein structure and function I gathered over years gave me a "warm start" in the company's research projects and quickly made me a key person responsible for protein design in several project teams. In the first half of my talk, I will focus on my latest academic work about unique voltage sensitivity of a heart channel HCN1, which opens upon membrane hyperpolarization unlike most voltagegated ion channels. To understand HCN1 "flipped" gating polarity we used extensive molecular dynamics simulations under hyperpolarizing conditions. The latter revealed a peculiar conformational change of the S4 helix (voltage sensor): it moved downward across the membrane and broke into two sub-helices located at an obtuse angle with respect to each other. Remarkably, the HCN1 structure published on the next day after our work confirmed the conformation of S4 under hyperpolarizing conditions predicted by the simulations. In the second half of my talk, I will touch upon one of the technological projects I've been working on when I started at Novo Nordisk. Small proteins combine best of two very different drug modalities: antibodies and small molecules. On the one hand, they can be more potent and specific than small molecules, and on the other hand, their production is significantly easier compared to antibodies. Identifying a proper scaffold for a small protein is crucial for binding to a target with a specific shape. To capture the variety of all natural scaffolds, we mined the Protein Data Bank and filtered small proteins with different shapes while yet having properties that make them a good starting point for further drug design. I will walk through our filtering pipeline with a focus on different properties important for a protein to be a drug candidate

Plenary Session 3, Tuesday, Sept 26, 10:00 *(Co-Host: Michael Netzer)*

Name / affiliation:: Vaibhavkumar S. Gawali, PhD, Charles River Laboratories

Title:Ionic and immune mechanisms of response to cancer immunotherapy and severe COVID-19

Authors: Vaibhavkumar S. Gawali*, Ameet A. Chimote, Hannah Newton, Martina Chirra and Laura Conforti

Affiliation: Division of Nephrology, Department of Internal Medicine, College of Medicine, University of Cincinnati, OH, United States.

Abstract: Ion channels in T cells control critical functions such as membrane potential, calcium influx, motility, and cytotoxicity. The defective function of calcium activated potassium channels (KCa3.1) and voltage-gated potassium channels (Kv1.3) in T cells of head and neck cancer (HNC) patients have been reported to contribute to the reduced immune-surveillance. In a translational study, on head and neck cancer patients we have studied the ionic mechanism of resistance to immunotherapy (immune checkpoint inhibitor- Pembrolizumab) focusing on characterizing the functional role of KCa3.1 and Kv1.3 channels in T cells. Fresh CD8+ cytotoxic T cells obtained from the peripheral blood of HNC patients (n = 42) and tumor samples were used to measure whole-cell K^+ currents using single-cell patch clamp electrophysiology. Intracellular calcium levels were measured using ratiometric Fura-2 dye and migratory abilities of T cells were measured using 3D-chemotaxis assay. The results of these functional studies show that compared to non-responders, HNC patients responding to immunotherapy showed increased activity of KCa3.1 and Kv1.3 channels, elevated intracellular calcium fluxes followed by increase in migratory ability of CD8+ T cells. These results provide strong evidence suggesting KCa3.1 and Kv1.3 channels regulate the response to cancer immunotherapy.

We have recently reported in a separate study, CD8+ T cells isolated from the peripheral blood of severely ill (ICU-hospitalization) COVID-19 patients treated with dexamethasone inhibited Kv1.3 channels resulting in reduced immune functions. Detailed analysis revealed that dexamethasone treatment in severe COVID-19 inhibited pro-inflammatory and immune exhaustion pathways, circulating cytotoxic and Th1 cells, interferon (IFN) signaling, genes involved in cytokine storm, and Ca^{2+} signaling. These results were also confirmed using *in vitro* treatment of dexamethasone on T cells obtained from healthy volunteers.

Overall, these two translational studies reports the critical role of KCa.31 Kv1.3 channels in regulating the response to cancer immunotherapy and severe COVID-19 providing a strong evidence of ion channels as a therapeutic drug targets in immune disorders.

Plenary Session 3, Tuesday, Sept 26, 10:25 (Co-Host: Michael Netzer)

Name / affiliation: Péter LUKÁCS, Centre for Agricultural Research, Martonvásár, Hungary

Title: Optimizing for Information Content on IonFlux Mercury Automated Patch Clamp

Abstract

*Peter Lukacs, Krisztina Pesti, Mátyás C. Földi, Adam V. Toth, and Arpad Mike**

Centre for Agricultural Research, Martonvásár, Hungary

In conventional pharmacology IC50 is everything. Ligands bind with high (typically nanomolar to low micromolar) affinity, using accurately arranged and oriented specific interactions (ionic interactions, hydrogen bonds, etc.). In sodium channel pharmacology IC50 is close to nothing, while conformational state-dependence and binding/unbinding kinetics are everything. For example if one measures the IC50 to be 100 µM in a certain experimental protocol, it may easily mean that the drug binds with 1 µM affinity for a millisecond, but then the channel gates into another conformational state, and the drug rapidly dissociates and for the next 100 millisecond it only has a 1000 µM affinity.

It is easy to see why this is advantageous in therapy: The drug that acts with a specific kinetics will be selective to a specific activity pattern, which might be the pattern of an unhealthy pathological cell causing arrhythmias, muscle spasms, neuropathic pain or epilepsy. Ideally each type of disease would need a drug with a unique kinetics.

We started to study the specific kinetic properties of individual drugs. We aimed to assess both the drug onset and offset kinetics (which occurs on the timescale of seconds), and the complex interaction between channel gating and drug binding (on the millisecond time-scale).

Standard high throughput screening projects using automated patch-clamp instruments often fail to grasp essential details of the mechanism of action, such as binding/unbinding dynamics and modulation of gating. Using the microfluidics-based automated patch clamp, IonFlux Mercury, we developed a method for a rapid assessment of the mechanism of action of sodium channel inhibitors, including their state-dependent association and dissociation kinetics.

To unravel this special interaction between the ion channel and the drugs, we designed a complex voltage protocol. This records the complete kinetic profile of each compound (state-dependent onset and offset kinetics, as well as its effect on the voltage dependence of steady-state inactivation) before, during, and after compound application at 1Hz time resolution.

Automated analysis of the results allows collection of detailed information regarding the mechanism of action of individual compounds. Our results show that the onset and the offset of drug effects are complex processes, involving several steps, which may occur on different time scales. This may help the assessment of therapeutic potential for hyperexcitability-related disorders, such as epilepsies, pain syndromes, neuromuscular disorders, or neurodegenerative diseases.

Plenary Session 4, Tuesday, Sept 26, 13:15 (Host: Lena Schwarz)

Stéphanie Baulac,

Paris Brain Institute (ICM)

Somatic mosaicism in epilepsy and cortical malformations

Postzygotic variants, or somatic variants, continuously occur along the zygote-to-adult developmental trajectory, resulting in genetic mosaicism. While most somatic mutations have no phenotype, some of them may contribute to generating normal neuronal diversity. In the central nervous system, brain mosaicism has been involved in the etiology of neurodevelopmental and neuropsychiatric disorders in humans, and is at the root of Focal Cortical Dysplasia (FCD) the most common focal cortical malformation associated with severe pediatric focal epilepsy. FCD type II (FCDII) is characterized by the focal disruption of cortical lamination and the presence of pathological cytomegalic cells, namely dysmorphic neurons (DN), that are thought to play a central role in the initiation of epileptic seizures.

Recent work has revealed the emergence of pathogenic somatic mutations occurring in early neuroglial progenitors during cortical development as significant causes of FCDII. The size of the FCD lesion is thought to primarily depend on the developmental stage at which the somatic mutation arises. Hence, the fraction of mutated cells varies from less than 1% of the resected cells in small FCDII to over 20% in hemimegalencephaly. Somatic mutations are typically found in the canonical PI3K-AKT-mTOR signaling cascade genes (*AKT3*, *DEPDC5*, *MTOR*, *PIK3CA*, *RHEB*, *TSC1*, *TSC2*), and lead to a cellular mosaic pattern of mTOR-hyperactive cells intermingled with normal-appearing neurons. Dysmorphic neurons carry the mTOR-activating mutations, establishing a causal link between the genetic mutation, hyperactivation of the mTOR pathway, and the generation of cytomegalic cells. The causal somatic mutation is thought to occur in a dividing cortical progenitor within the ventricular zone during corticogenesis which is then transmitted to the clonal daughter cells. A small lesion would therefore reflect a late-occurring somatic mutation with a small postmitotic progeny, while a large lesion, as in hemimegalencephaly (HME), would reflect an early-occurring mutation affecting a large postmitotic progeny. How activation of the mTOR pathway relates to epileptogenesis and seizures is unclear, as this signaling cascade is involved in several physiological processes, from metabolic response to extracellular nutrients and growth factors (Liu and Sabatini, 2020). In utero-based electroporation animal models modeling somatic mutations can recapitulate some aspects of the neuropathological and clinical features of FCDII and hence, provide a better understanding of associated molecular alterations involved in the emergence of this mosaic disorder.

Plenary Session 4, Tuesday Sept 26, 13:40 (Co-Host: Nathalie Agudelo-Duenas)

Name / affiliation: Jasmin MORANDELL, University orf Trento

Title: *circHTT*, a novel circular RNA molecule from the Huntington's disease gene locus: functional characterization and pathophysiological implications

J. Morandell[1], , A. Monziani[1], J. Döring[1], C. Oss Pegorar[1], A. Mattiello[1], G. Bergonzoni[1], T. Tripathi[1], F. Di Leva[1], E. Kerschbamer[1], D. Donzel[2], V.B. Mattis[3], J. Rosati[4], C. Dieterich[5], E. Dassi[1], V.C. Wheeler[6], H. Hansíková[7], Z. Ellederová[8], G. Viero[2], J.E. Wilusz[9], M. Biagioli[1]

[1] NeuroEpigenetics Laboratory, Dept. Cellular, Computational and Integrative Biology - CIBIO, Univ. of Trento, Trento, Italy
[2] Institute of Biophysics Unit at Trento, National Research Council – CNR, Trento, Italy
[3] Board of Governor's Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, USA
[4] Mendel Laboratory, IRCCS Casa Sollievo della Sofferenza, Rome, Italy
[5] Section of Bioinformatics and Systems Cardiology, Univ. Hospital Heidelberg, Heidelberg, Germany
[6] Molecular Neurogenetics Unit, Center for Genomic Medicine, Massachusetts General Hospital, Boston, USA
[7] Dept. Pediatrics and Adolescent Medicine, First Faculty of Medicine, Charles Univ. and General Univ. Hospital, Prague, Czech Republic
[8] Research Center PIGMOD, Institute of Animal Physiology and Genetics, Czech Academy of Science, Libechov, Czech Republic
[9] Dept. Biochemistry & Biophysics, Univ. of Pennsylvania Perelman School of Medicine, Philadelphia, USA

Circular RNAs (circRNAs), single-stranded, circularized RNA molecules, are particularly enriched in neurons and their functional relevance for brain development and neurological disorders has become increasingly evident in recent years. Here, we identified and confirmed the first circular RNA stemming from the human *HTT* locus, *circHTT*, and showed its conservation in mouse (*circHtt*) and mini-pig. We validated the circularity of the identified molecules by divergent primer amplification, sequencing and RNase R treatment. Then, we analysed the expression pattern of *circHTT*/*circHtt* in human and mouse tissues and revealed ubiquitous expression of the molecule with highest levels in the mature brain, in line with circular RNA characteristics. To identify possible implications for Huntington's Disease (HD), we studied its expression pattern in induced pluripotent stem cell (iPSC)-derived neuronal cells and HD mouse models. We found that *circHtt*/*circHtt*expression augments significantly with increasing number of CAG repeats in terminally differentiated cortical neurons, and in brain tissue of HD mouse models. Analysis of primary cortical neurons from E17.5 zQ175 mouse embryos furthermore indicated that the increased *circHtt* levels in the HD model arise upon onset of neuronal differentiation. These findings strongly suggest possible implications for HD pathology. Analysis of the *circHtt* sequence revealed binding sites for RNA binding proteins, and the presence of a putative IRES sequence, suggesting a potential association of *circHTT*/*circHtt* with ribosomes. Indeed, polysome fractionation experiments on brain tissue indicate an association of *circHtt* with the small ribosomal (40S) subunit. To further elucidate the biological function(s) of this molecule, knock-down and over-expression studies are currently ongoing. In conclusion, we uncovered a circRNA sensitive to the HD mutation, which may be relevant for HD pathophysiology and/or to modulate huntingtin expression. Thus, it is tempting to speculate that *circHTT* may represent a novel, urgently needed, molecular drug target for Huntington's Disease.

Plenary Session 4, Tuesday Sept 26, 14:05 (Co-Host: Nathalie Agudelo-Duenas)

Name / affiliation: Julia Michalska, Scientist Optical Connectomics E11BIO

Title: A versatile toolbox for the comprehensive analysis of nervous tissue organization with light microscopy

Abstract:

The brain is an exceptionally sophisticated organ consisting of billions of cells and trillions of connections that orchestrate our cognition and behavior. To decode its complex connectivity, it is pivotal to disentangle its intricate architecture spanning from cm-sized circuits down to tens of nm-small synapses. To achieve this goal, we have developed CATS - Comprehensive Analysis of nervous Tissue across Scales, a toolbox for obtaining a holistic view of nervous tissue context with fluorescence microscopy. CATS combines comprehensive labeling of the extracellular space with information on molecular markers, super-resolved data acquisition and machine-learning based data analysis for segmentation and annotation. We show that CATS can be used to analyze key features of nervous tissue connectivity, ranging from whole tissue architecture, neuronal in- and output-fields, down to synapse morphology

Poster Session, Monday Sept 25, from 15:50 (flash talks Host: Gerhard Ecker)

POSTER #1

Name / affiliation: Nathalie Agudelo-Dueñas, Institute of Science and Technology Austria (ISTA)

Title: Visualizing the transcriptional landscape with tissue context

Abstract

Nathalie Agudelo-Dueñas*[*1]*,** *Julia Lyudchik[1], Caroline Kreuzinger[1], Mojtaba R. Tavakoli[1], Giulio Abagnale[2], Julia M. Michalska[3], Christoph Sommer[1],* ***Johann G. Danzl[1]

[1] Institute of Science and Technology Austria (ISTA), [2] St. Anna Children's Cancer Research Institute(CCRI), [3] E11 Bio

Biological systems are intrinsically heterogeneous, from the level of molecular arrangements and interactions to whole tissue organization. To understand the complexity of these systems, it is fundamentalto study them in their native context, which requires assessing their intricate structure and function in a spatially informed manner. Over the last decade, there has been a rapid advancement in the field of *spatial omics*, especially at the transcript level measuring gene expression, which has been instrumental in understanding how mRNA distribution and abundance define cell identity and function. This project aimsto develop a highly multiplexed and modular methodology for integrated structural and multi-molecular characterization, as a means to visualize the spatial arrangement of the transcriptome with subcellular to tissue context. Given the importance of the compartmentalized organization of mRNAs (*local transcriptome*) in neurons, we apply a 242-gene panel to target neuron-specific transcripts in mouse brain tissue via Multiplexed Error Robust FISH (MERFISH). Importantly, we have adapted our protocols to work with thicker sections and gain 3D MERFISH spatial information. We also combine the transcriptional information with a morphological readout based on labeling the extracellular domain, which provides us with richer contextual information and allows us to locate mRNAs within distinct neuronal compartmentsat subcellular resolution. We envision that this technology will enable a more accurate characterization ofthe local transcriptome, to achieve a better understanding of how neurons respond to their functional
demands in both health and disease.

POSTER #2

Name / affiliation: Sergio Armentia Matheu, Institute of Organic Chemistry, University of Vienna

Title: Development of Novel Fluorescent Dyes in the Making of New Biologically Active Conjugates

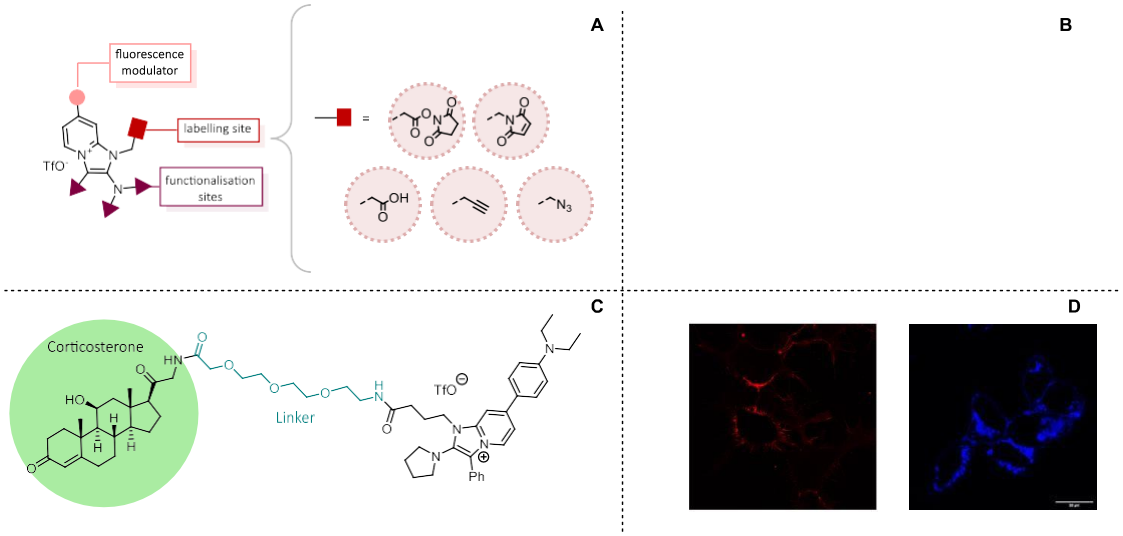
Sergio Armentia Matheu[1], Oliver Belleza[2], Yi Xiao[1], Nina Kastner[2], Iakovos Saridakis[1], Stefanie Rukavina[1], Margaux Riomet[1], Harald Sitte[2], Nuno Maulide[1]

1 Faculty of Chemistry, Institute of Organic Chemistry, University of Vienna

2 Center for Physiology and Pharmacology, Institute of Pharmacology, Medical University of Vienna

Synthetic fluorescent dyes have become indispensable tools in chemical biology: their development over the years has allowed the elucidation of key biochemical and cellular pathways.[1,2] Our group has developeda promising and easily available novel class of fluorescent dyes based on an imidazo[1,2-a]**pyr**idinium trif**late** scaffold, which we term PyrAtes (**Fig. A**).[3] These present a moiety in ortho from that pyridinium center that allows the modulation of the fluorescent properties – changing absorbance and emission with abroad stokes shift. Importantly, they present a versatile labelling site (**Fig. A**) which, by covering a broadrange of functionalities, allows selective coupling with other active compounds while preserving the fluorescent properties intact.

Here we present PyrAtes as a versatile tool to generate labelled conjugates of biological interest. We successfully derivatized such core with bio-active compounds like citalopram (**Fig. B**) or corticosterone (**Fig. C**). In addition, the PyrAte conjugate has allowed successful labeling of living cells expressing serotonin transporters (SERT) or organic cation transporters (OCT1 and OCT3), their respective targets (**Fig. D**).[4]



References:

(1) J. Jun, D. Chenoweth, E. Peterson, *Org. Biomol. Chem.*, **2020**, 18, 5747-5763; (2) V. Martynov et al., *Acta Nature*, **2016**, 8, 33-46; (3)Maulide *et al.*, *J. Am. Chem. Soc.*, **2023**, submitted; (4) Maulide *et al.*, **2023**, manuscript in preparation

POSTER #3

Name / affiliation: Bozhidar Baltov, University of Vienna

Title: Assay for evaluation of proarrhythmic effects of herbal products

Stanislav Beyl, Igor Baburin, Jakob Reinhardt, Phillip Szkokan, Aleksandra Garifulina, Eugen Timin, Udo Kraushaar, Olivier Potterat, Matthias Hamburger, Philipp Kügler, Steffen Hering

Abstract: Regulatory guidelines for drug development warrant a reduced risk of arrhythmia-related side effects. Despite ample evidence for the presence of hERG inhibitors and other ion channel blockers in herbal drugs and phytomedicines, the regulation of their evaluation and use is not rigorously mandated by safety and marketing authorities. The main aim of this study was to propose a cardiac safety paradigm for the evaluation and classification of proarrhythmic properties of plant extracts, based on experimental approaches comprised in the Comprehensive *In vitro* Proarrhythmia Assay (CiPA). We implemented essential CiPA experimental approaches to study the potent hERG inhibitors of Evodia rutaecarpa: dehydroevodiamine (DHE) and hortiamine. Proarrhythmic effects of 12 Evodia preparations, containing different amounts of the two alkaloids, were analysed.

Microelectrode array studies (MEA) and voltage sensing optical (VSO) technique on human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were combined with patch clamp ionic currentmeasurements in mammalian cell lines expressing Nav1.5, Cav1.2, and hERG, *in silico* simulations of action potentials (APs) and statistic regression analysis. Modelling of the APs was conducted with the O'Hara-Rudy model and the multiple ion channel effects (MICE) model.

AP prolongation and early afterdepolarisations (EADs) in hiPSC-CMs correlated with DHE/hortiamine content of the 12 tested extracts, as observed with VSO technique. The content of the major proarrhythmicalkaloid of Evodia – DHE, ranged from 0.45 mg/g to 5.97 mg/g. Based on the effects of 10 µg/ml on APs the extracts could be divided into low- (ΔAPD90 <50 %), intermediate- (ΔAPD90 >50 %), and high- arrhythmogenic (AP oscillations). Furthermore, the effects of the extracts were analysed with planar patchclamp and revealed concentration-dependent inhibition of potassium currents through hERG. Further evidence of drug-induced QT prolongation was acquired with the MEA technology. A concentration- dependent increase in the field potential duration up to 50.2 % (DHE) and 104.3 % (hortiamine) was observed while a significant inhibition of the amplitude occurred only at the highest concentration (300 nM). *In silico* simulations reproduced EADs and torsadogenic effects and the high torsadogenic risk predicted was comparable to known torsadogenic drugs.

While significant data sets for cardiotoxic drug effects on APs of hiPSC-CMs have been generated to classify proarrhythmic events, such studies for herbal drugs are currently missing. Taken together we propose an *in vitro* assay for the estimation of the proarrhythmic potential of Evodia and herbal extracts in general.

POSTER #4

Name / affiliation: Oliver Belleza / Institute of Pharmacology, Medical University of Vienna

Title: Novel fluorescent probes for imaging the serotonin transporter

Abstract:

Nina Kastner, Kathrin Jäntsch, Harald Sitte (Institute of Pharmacology, Medical University of Vienna)
Iakovos Saridakis, Miran Lemmerer, Margaux Riomet, Yi Xiao, Sergio Armentia Matheu, Saad Shaaban, Nuno Maulide (Institute of Organic Chemistry, University of Vienna)
Nadja Singer, Pedro Sánchez-Murcia, Leticia González (Institute of Theoretical Chemistry, University of Vienna)Xavier Westergaard, David Sulzer (Department of Psychiatry, Columbia University/United States)

The serotonin transporter (SERT) belongs to a family of transmembrane proteins expressed in the nervoussystem that facilitates the cellular transport of biogenic monoamine neurotransmitters. These proteins areimportant drug targets for the treatment of mental and behavioral disorders such as depression and attention-deficit hyperactivity disorder. Therefore, compounds that can serve as pharmacological tools to investigate the activity of these transporters are clinically relevant. Herein, a recently described class of fluorophores called PyrAtes were developed into fluorescently labeled ligands that can bind to SERT. Twocompounds each with a PyrAte fluorophore attached to (*S*)-citalopram, a selective serotonin reuptake inhibitor (SSRI), via either a six-carbon chain linker (MILE753) or a three-carbon chain linker (IASA554)were synthesized and characterized. Docking and molecular dynamics experiments were performed to observe their binding affinities to SERT. The activity of these resulting compounds was further confirmedin HEK293 cells overexpressing SERT using cell-based radioligand uptake assays and confocal microscopy. Additionally, their utility in imaging endogenously expressed transporters was explored *ex vivo* in acute mouse brain slices using two-photon microscopy. The inhibitory activity of (*S*)-citalopram was reduced by the PyrAte tag by around 10- to 20-fold, with IASA554 and MILE753 possessing IC50 values of 0.40 and 0.83 µM, respectively. These compounds, however, were nevertheless effective and specific in fluorescent labelling of SERT. Our results not only confirm the utility of such compounds in the fluorescence imaging of transporters, but also provide insights into ways of improving the design of PyrAte fluorophores and their fluorescent drug conjugates in the future.

POSTER #5

Name /affiliation: Bogdan-Razvan Brutiu[a] / ([a]University of Vienna, Institute of Organic Chemistry, Maulide Group)

*Iakovos Saridakis[a], Thomas Leischner[a], Saad Shaaban[a], Manuel Matzinger[b], Fränze Müller[b], Micha, Birklbauer[c], Viktoria Dorfer[b,] *, Karl Mechtler[c,] *, Nuno Maulide[a,] **

Title: New enrichable cross-linkers for protein-protein interaction analysis by mass spectrometry

Abstract - Chemical cross-linking mass spectrometry (XL-MS) has emerged as an alternative and complementary tool for mapping interaction sites within protein complexes and to obtain low-resolution native structure information with minimal interference.[1] In recent years, a multitude of new cross-linker molecules, enrichment strategies, MS-methods for data acquisition and software tools for data interpretation have been developed, which highlights the rising importance of XL-MS.

One of the most utilized crosslinkers nowadays is DSSO (disuccinimidyl sulfoxide).[2] Despite its application in a variety of protein analysis, the low chemical stability remained a drawback. Therefore, weaimed to synthesize a more stable, yet still reactive cross-linkers bearing an enrichment cite.

Herein, we present our results of a study culminating with the synthesis of new XL reagents, as well asthe ongoing analysis using algorithms capable of analyzing peptides cross-linked with different cross-

linkers and possible future applications within living cells (Figure 1).[3]

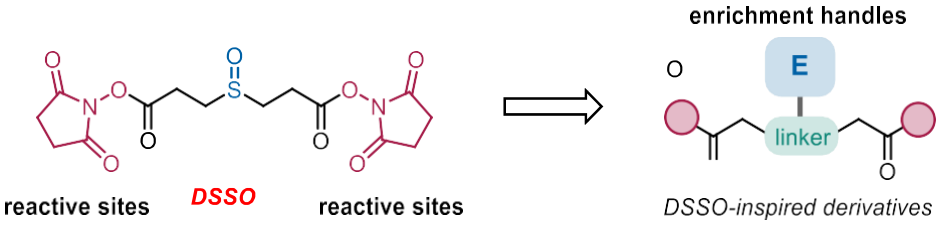


Figure 1. DSSO-inspired novel class of MS-linkers.

References

[1] Z. Orbán-Németh, *et al*. *Nat. Protoc.* **2018**, *13*, 478–494.

[2] A. Kao, *et al*. *Mol. Cell Proteomics* **2011**, *10*, M110.002212.

[3] Manuscript in preparation.

Name / affiliation: Theres Friesacher / University of Vienna, Division of Pharmacology and Toxicology; Weinzinger group

Title: Ethosuximide inhibition of GIRK channels

Abstract

Boris Shalomov[1], Theres Friesacher[2], John Carlo Combista[1], Haritha P. Reddy[1], Daniel Yakubovitch[1],Shoham Dabbah[1], Eva-Maria Zangerl-Plessl[2], Anna Stary-Weinzinger[2], Nathan Dascal[1]

1 Department of Physiology and Pharmacology, School of Medicine, Tel Aviv University

2 Department of Pharmaceutical Sciences, Division of Pharmacology and Toxicology, University ofVienna

G-protein coupled inwardly rectifying potassium (GIRK) channels are key players in inhibitory neurotransmission in the heart and brain. There are 4 subfamilies of GIRK channels (GIRK1-4). Their activation is complex and involves a variety of factors, such as the membrane component phosphatidylinositol 4,5-bisphosphat (PIP_2) and the Gβγ subunit of the G protein. Mutations in GIRK channels are associated with cardiovascular and neurological conditions.

Ethosuximide (ETX) is an antiseizure drug known to inhibit GIRK channels and therefore has therapeuticpotential for diseases caused by gain-of-function GIRK mutations. We investigate GIRK inhibition by ETX using electrophysiology experiments and µs-long molecular dynamics (MD) simulations. We showthat the effect of ETX is allosteric. Furthermore, the inhibition is potentiated when the channel is activatedby the Gβγ subunit of the G-protein. MD simulations of a GIRK2 homotetramer in the presence of ETX reveal a stable drug binding pose close to the binding site of the activator PIP_2. Drug binding leads to the displacement of PIP_2 and closing of the channel. The binding pose is corroborated by electrophysiology experiments with GIRK2 mutants.

POSTER #7

Name / affiliation: Aleksandra Garifulina, University of Vienna, Department of PharmaceuticalSciences

Title: β subunits of $GABA_A$ receptors form proton gated ion channels

Abstract

Aleksandra Garifulina[1], Theres Friesacher[1], Marco Stadler[1], Eva-Maria Zangerl-Plessl[1], Margot Ernst[2], Anna Stary-Weinzinger[1], Anita Willam[1,3], Steffen Hering[1,3]

[1] Department of Pharmaceutical Sciences, University of Vienna, A-1090 Vienna, Austria

[2] Department of Pathobiology of the Nervous System, Medical University of Vienna, A-1090 Vienna, Austria

[3] ChanPharm GmbH, Am Kanal 27, Top 2/3/5, 1110, Vienna, Austria

$GABA_A$ receptors are pentameric ligand-gated anion channels (pLGIC) permeable to chloride and bicarbonate. These receptors mediate fast signal transmission in the central nervous system (CNS) as well as periphery. While the *Gloeobacter violaceus* ion channel (GLIC) is a prokaryotic pLGIC homologue being gated upon proton application, protons as agonists have not been shown to activate $GABA_A$ β receptors. We describe, for the first time, direct activation of homopentameric $GABA_A$ β3, β2 and β1(S265N) receptors by protons in a concentration-dependent manner (pH_{50} is in the range 6- 6.3). Steady-state activation and desensitization of β-homomeric $GABA_A$Rs revealed significant window currents at physiological pH suggesting the possibility that a significant fraction sojourns in an open state.

In order to identify the location of putative proton activation site(s) we mutated the protonable residues inthe pore forming transmembrane region (TM2) of β3 $GABA_A$R. Mutation of H267A completely prevented channel activation by acidification and, simultaneously, induced significant picrotoxin sensitive baseline currents indicating an increased open probability. Furthermore, the introduction of histidine (G331H) and glutamate (A334E) in homologous positions of the proton insensitive $GABA_A$R ρ1 subunit transfers proton-dependent gating, thus highlighting the role of this interaction in proton sensitivity. This is also supported by molecular dynamics simulations indicating that protonation of H267 increases formation of hydrogen bonds between E270 and H267 leading to a pore stabilising ring formation and accumulation of Cl^- within the transmembrane pore. Deprotonation of H267 is accompanied by a general broadening of thetransmembrane pore and expulsion of Cl^-.

Our findings warrant studies on the molecular base of proton dependent gating and a potential physiological and pathophysiological impact of this novel channel type.

POSTER #8

Name / affiliations: Ralph Gradisch / Medical University of Vienna, Institute of Physiology and Pharmacology

Title: Ligand coupling mechanism of the human serotonin transporter differentiates substrates from inhibitors

Abstract

*[1]Ralph Gradisch, [2]Katharina Schlögl, [1]Erika Lazzarin, [1,3]Marco Niello, [1]Julian Maier, [4,5,6]Felix P. Mayer, [1]Leticia Alves da Silva, [1]Sophie MC Skopec, [4,5]Randy D Blakely, [1]Harald H Sitte, [2]Marko D Mihovilovic, [1]Thomas Stockner**

Affiliations:

[1] Medical University of Vienna, Institute of Physiology and Pharmacology,
[2] TU Wien, Institute of Applied Synthetic Chemistry,
[3] Genetics of Cognition Laboratory, Neuroscience area, Istituto Italiano di Tecnologia,
[4] Florida Atlantic University, Department of Biomedical Science, Brain Institute
[5] Stiles-Nicholson Brain Institute, Jupiter, FL 33458, USA
[6] Department of Neuroscience, Faculty of Health and Medical Sciences, University of Copenhagen

The presynaptic serotonin (5HT) transporter (SERT) clears extracellular 5HT following vesicular release to ensure temporal and spatial regulation of serotonergic signalling and neurotransmitter homeostasis. Clinically approved drugs used for the treatment of neurobehavioral disorders, including depression, anxiety, and obsessive-compulsive disorder that target SERT trap the transporter in the outward-open state thus blocking the transport cycle. In contrast, illicit drugs of abuse like amphetamines reverses SERT directionality, thereby causing 5HT efflux. Both result in an increase of extracellular 5HT levels.Stoichiometry of the transport cycle has been described by kinetic schemes, whereas the structures of the main conformations provide only static coordinates of molecular features of the process. By combining in-silico molecular dynamics modelling approaches with in-vitro and ex-vivo biochemical experiments and making use of a homologous series of 5HT analogues, we uncovered the essential coupling mechanism between the substrate and the transporter triggering the uptake process. The free energy calculations showed that only scaffold-bound substrates can correctly close the extracellular gate. Attractive forces acting on the bundle domain through long-range electrostatic interactions tilt the bundle domain towards the scaffold domain. The associated spatial requirements define substrate and inhibitor properties, enablingnew possibilities for rational drug design approaches.

POSTER #9

Name / affiliation: Nejra Granulo[1,2], Gerhard F. Ecker[1]

[1] University of Vienna, Department of Pharmaceutical Sciences, Josef Holaubek Platz 1

[2] University of Vienna, Research Platform NeGeMac – Next Generation Macrocycles to Address Challenging Protein Interfaces

Title: Macrocycles as potential inhibitors of GLUT1 and GLUT4

Solute Carrier Transporters (SLC) represent the membrane-bound proteins that control the transport of broad scope of substrates across biological membranes. They are directly involved in physiological processes and the maintenance of vital functions of a single cell.

SLC2 family transporters (also known as the GLUT family) have already been explored as a therapeutic drug class since they are key regulators of glucose metabolism. While glucose is necessary for the growth, proliferation, and differentiation of cells, cancer cells exhibit altered glucose metabolism compared to normal cells. The increased glucose consumption by cancer cells is pivotal for their survivaland growth, presenting a strategic opportunity for targeted antitumor therapies. In recent years, the development of drugs affecting the energy intake of tumor cells has become a research hotspot. GLUT inhibitors are gaining increased attention because they can block the energy supply of malignant tumors. Among the GLUT family, GLUT1 and GLUT4 are considered high-affinity transporters. GLUT1 is highly expressed in breast cancer cells, while GLUT4 has been reported in multiple myeloma.

Macrocycles have already been used successfully as antitumor drugs. Their ability to accommodate the facet of the binding site lies in their adaptability and controlled flexibility, allowing them to address protein-protein interaction at low entropic cost and define them as tool compounds of interest for the drug targets.

We identified two macrocyclic scaffolds and generated two compound libraries (for each scaffold). Usingthe crystal structure of GLUT1 (PDB: 5EQI) and GLUT4 (PDB: 7WSN), we generated two molecular docking protocols for both compounds' libraries. The analysis of the docking results, combined with the extensive visual inspection of interactions in the binding pocket resulted in the identification of potential inhibitor candidates for both GLUT1 and GLUT4. The potential candidates make key interactions in the binding pocket, (GLUT1: T137, H160, Q282, W388, N411, W412; GLUT4: S153, N176, Q298, W404,

N427, W428) which have been reported in the literature for Cytochalasin B that inhibits both transporters. Furthermore, three candidates coming from the Scaffold 1 compounds' library have been amongst the top-ranked for both transporters, suggesting their possible potential as dual-action agents.

POSTER #10

Name / affiliation: Palle Steen Helmke/ Department of Pharmaceutical Sciences, University of Vienna

Title: Systemic fingerprints for predicting liver related adverse events

Palle Helmke[a], Barbara Füzi[a], Gerhard F.Ecker[a]

[a]Department of Pharmaceutical Sciences, University of Vienna

Abstract: Predicting a complex endpoint such as hepatotoxicity/DILI (Drug-induced liver injury) or cholestasis is not a straightforward task. Accordingly, it is crucial to understand the cellular mechanisms leading to an adverse event. The involved biological pathways are defined as a series of actions among molecules withina cell that ultimately result in an alteration or particular product. This is depicted using systems biology approaches. Thus, the aim was to connect drugs to biological pathways, through proteins the compound alters, to understand their systemic effects. This knowledge is essential to predict an adverse event.

The following characterization of compound-interaction profiles was carried out using the open-source publicly available software KNIME utilizing a workflow called "Path4Drug" [1]. In a first step, drug-targetpairs were retrieved from 5 publicly available databases (Drugbank, ChEMBL, IUPHAR/BPS, PharmGKBand TTD) to depict compound-target interactions. Hence, target proteins were identified for a compound that were labelled as active in a biological assay and tissue specific target profiles were created with The Human Protein Atlas. Finally, to connect a drug to a systemic effect, the targets were annotated to pathways using Reactome pathway analysis API service performing a statistical overrepresentation test. As an output, two binary matrices for the compound-target and compound-pathway interactions were obtained with 1 indicating an interaction and 0 representing no interaction. These matrices served as an input for arandom forest classifier (RF). As a first use case the Drug Indued Liver Injury (DILI) Rank dataset providedby the FDA was applied, consisting of drugs causing DILI and drugs without a DILI concern. Using the pathway profiles as descriptor for the RF, an accuracy of 0.65 was achieved with the pathways fatty acid metabolism and arachidonic acid metabolism as top descriptors in a feature importance exercise. In a nextstep, the described approach will also be conducted for 578 compounds of a cholestasis (reduced or stoppedbile flow) dataset.

1. Füzi, B; Gurinova, J; Hermjakob, H; Ecker, GF; Sheriff, R Front. Pharmacol. Sec. Predictive Toxicology, Volume 12, 2021, 1-11

POSTER #11

Name / affiliations: Jiahui Huang, University of Vienna, Pharmacoinformatics Research Group

Title: ProteoMutaMetrics: Machine Learning Approaches for SLC6 Mutation Pathogenicity Prediction

Abstract:

Jiahui Huang[1], Tanja Osthushenrich[2], Aidan MacNamara[2], Anders Mälarstig[3], Silvia Brocchetti[4], Samuel Bradberry[4], Lia Scarabottolo[4], Evandro Ferrada[5], Sergey Sosnin[1], Daniela Digles[1], Giulio Superti-Furga[5],Gerhard F. Ecker[1*]

[1] *University of Vienna, Department of Pharmaceutical Sciences, Vienna, Austria*

[2] *Bayer AG, Division Pharmaceuticals, Biomedical Data Science II, Wuppertal, Germany*

[3] *Emerging Science & Innovation, Pfizer Worldwide Research, Development & Medical, USA*

[4] *Axxam SpA, Bresso (Milan), Italy*

[5] *CeMM, Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria.*

The Solute Carrier transporter family 6 (SLC6) is of key interest for their critical role in transport of smallamino acids or amino acid-like substances. Their dysfunction is strongly associated with a few human diseases, including schizophrenia, depression, and Parkinson disease. Linking single point mutations to disease may support insights into the structure-function relationship of these transporters. The aim of thiswork was to predict the potential pathogenic effect of single point mutations in the SLC6 family. Missensemutation data was retrieved via a KNIME workflow from three databases (UniProt, LitVar, ClinVar) covering multiple protein-coding transcripts with protein residue level assignment. Subsequently, single point mutations were transferred into the canonical sequence or sequence variations yielding putative mutated sequences. Finally, the sequences were cut into twelve domains defined according to thetransmembrane domain (TMD) of the SLC6 transporters. As encoding for machine learning models, physicochemical descriptors were used to calculate overall sequence properties for both original and mutated sequences. The pathogenicity labels were transformed into binary vectors based on the descriptions from the data sources. We built several classification models, namely Support Vector Machine(SVM), Logistic Regression (LR), Random Forest (RF), and Extreme Gradient Boosting (XGBoost) withthe hyperparameters optimized through Grid Search. We estimated the model performance using repeatedstratified k-fold cross-validation. Mean accuracy values of the generated models are in the range of 0.8 with cross validation and parameter tuning. Analysis of feature importance points towards distinct regionscross the SLC6 transporter family where mutations increase the risk for pathogenicity. When applying themodel on an independent validation set, the performance in accuracy dropped to averagely 0.6 with high precision but low sensitivity scores.

POSTER #12

Name / affiliations: Nina Kastner, Medical University of Vienna, Institute of Pharmacology

Title: Characterization of MDMA derivative 1,3-Benzodioxolylbutanamine (BDB) and its structural analogs

Abstract

Nina Kastner[1], Julian Maier[1], Marion Holy[1], Fatemeh Kooti[1], Bruce E Blough[2], Joseph Wilson[2], Kevin Murnane[3], Harald H. Sitte[1,4]

1 *Institute of Pharmacology, Medical University of Vienna, Vienna, Austria*
2 *Research Triangle Institute, Center for Drug Discovery, Research Triangle Park, NC, USA*
3 *Louisiana State University Health Sciences Center, Dept. of Pharmacology Toxicology & Neuroscience and Louisiana Addiction Research Center, Shreveport, LA, USA*
4 *AddRess Centre for Addiction Research and Science, Medical University of Vienna, Vienna, Austria*

3,4-Methylenedioxymethamphetamine (MDMA) and its structural analog 3,4-methylenedioxyamphetamine (MDA) are commonly abused for recreational purposes due to their psychostimulatory effects. Pharmacologically, they are characterized by acting as substrates and releasersat the monoamine transporters for dopamine (DAT), norepinephrine (NET) and serotonin transporter (SERT). Structurally similar illicit derivatives of these substances are constantly appearing on the street markets and additionally, MDMA is being investigated as possible adjuvant drug in psychotherapy to treatdepression and posttraumatic stress disorder. While MDMA proves to be a valid drug to treat these disorders, there still remains the lingering question of abuse liability and cytotoxicity. To address these questions and concerns, it is necessary to investigate the interactions of MDMA and the herein described related compounds with monoamine transports and receptors.

In this study, we characterized the MDMA derivative 1,3-Benzodioxolylbutanamine (BDB) and its structural analog N-Methyl-1,3-Benzodioxolylbutanamine (MBDB) and compared them to the well-established MDA and MDMA. The compounds differ in the addition of a methyl substituent on the terminalamine group and/or increased carbon chain length the α-carbon. To evaluate interactions with monoamine and uptake-2 transporters, we performed in vitro radiotracer uptake inhibition and superfusion experimentsin human embryonic kidney 293 (HEK293) cells stably expressing the human isoform of the respective transporter.

We observed that that the interaction profile of BDB compares very well to MBDB and MDA at SERT andDAT, but slightly differs at NET. Additionally, due to the higher selectivity for DAT than SERT of BDB,MBDB and MDA, they may be associated with higher abuse liability than MDMA. However, in contrastto BDB and MBDB, MDA and MDMA evoked substrate release at DAT, which is also linked with abuse.Further, BDB and MDA were less potent inhibitors at OCT1 than MDMA and MBDB, while showing more interaction at OCT3. To further characterize the substances, we plan to investigate releasing properties at NET and the potential interactions with serotonin (5-HT) receptors, which are known to have a contributing factor in the stimulant effects of MDA and MDMA.

POSTER #13

Name / affiliation: Michael A. Netzer, Division of Pharmacology & Toxicology, University of Vienna

Title: Electrophysiological characterization of cardiac organoids

Abstract:

Authors:
Michael A. Netzer[1,2], Alison Deyett[3], Clara Schmidt[3], Simon Haendeler[4], Lokesh Pimpale[5], SashaMendjan[3] and Steffen Hering[1,2]

1 - Division of Pharmacology and Toxicology, Department of Pharmaceutical Sciences, University ofVienna, A-1090, Vienna, Austria

2 - ChanPharm GmbH, Am Kanal 27, Top 2/3/5, 1110, Vienna, Austria

3 - Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna, Austria

4 - Center for Integrative Bioinformatics Vienna (CIBIV), Max Perutz Laboratories, University ofVienna and Medical University of Vienna, Vienna Bio Center (VBC), Vienna, Austria

5 - HeartBeat.bio, Vienna, Austria

The number one cause of human fetal death are defects in heart development. Because the embryonic heartis inaccessible and no chamber specific *in vitro* models exist, determining the causes of disease is difficult.Therefore, a human cardiac organoid platform was recently established recapitulating the development ofall major embryonic heart compartments, including right and left ventricles, atria, outflow tract, and atrioventricular canal[1,2]. Here, we characterize the electrophysiological properties of these cardiac organoids. Calcium signaling was evaluated using genetically encoded GCaMP6f reporter lines, and actionpotentials were analyzed by single cell patch clamp as well as 3D optical measurements. Together, the datarevealed embryonic electrophysiological properties of cardiac organoids.

1 - Hofbauer, Pablo, et al. "Cardioids reveal self-organizing principles of human cardiogenesis." *Cell*

184.12 (2021): 3299-3317.

2 - Schmidt, Clara, et al. "Multi-chamber cardioids unravel human heart development and cardiac defects."

bioRxiv (2022).

POSTER #14

Name / affiliation: Abir Omran / University of Vienna, Pharmacoinformatics Research Group

Title: Analysing the Co-existence of Immune Reaction and Liver Toxicity for Therapeutical Monoclonal Antibodies

Abir Omran[1], Barbara Füzi[2], Alexander Amberg[2], Gerhard F. Ecker[1]
[1] University of Vienna, Department of Pharmaceutical Sciences, Josef-Holaubek-Platz 2, Vienna, Austria
[2] Sanofi, Department of Preclinical Safety, Frankfurt, Germany

Drug-induced liver injury (DILI) refers to several adverse events associated with the liver. There are different mechanisms of action causing DILI; for small molecules, it's usually caused by their metabolites. However, this is not the case for larger molecules such as monoclonal antibodies (mAbs); these drugs are administrated differently and are subject to different drug metabolism. Certain liver toxicities caused by mAbs can be connected to their effect on the immune system. The association between monocytes and non-alcoholic fatty liver has been reported by previous studies. In this work, weused post-marketing data to analyse the co-existence of immune reaction and liver toxicity for therapeutical mAbs.

The state of the immune system during the immunotherapy treatment was described by using adverse event endpoints connected to white blood cells as features. The number of unique liver toxicity endpointswere counted for every mAb, a threshold was set to define the positive and negative class. Due to imbalanced classes, an undersampling approach was performed by removing data points from the positive class. A random forest model was built to perform a feature importance; UMAP was used to cluster the data with the most informative features.

A clear separation in the UMAP was seen between the classes with very few outliers.

POSTER #15

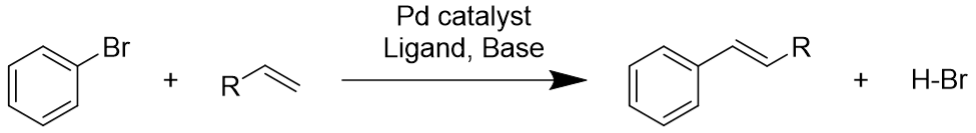
Name / affiliation: Eleni Papaplioura / TU Wien, Institute of Applied Organic Chemistry

Title: Substituting Gaseous Reagents for Solid Alternatives

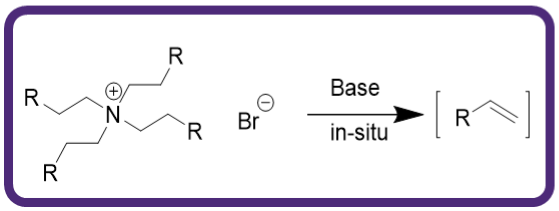
Supervisor: Michael Schnürch

Co-supervisor: Marko D. Mihovilovic

Introduction of short carbohydrate chains are challenging functionalization reactions due to low reactivityof alkylhalides and inconvenient use of short chain olefins as alkylating/alkenylating agents. This project is centered on the development of a convenient Heck vinylation protocol that circumvents the use of ethylene gas. Olefins and especially arylethens with their tunable electronic and steric properties are important precursors for the synthesis of bioactive compounds as well as polymers. However, conventionalmethodologies utilize olefins that are gaseous at room temperature or elaborate high pressure equipment. A sustainable and safe approach that can tackle this issue involves the use of solid and easy to handlesources of alkenes and in situ generation of the required reactive coupling partners as illustrated by the useof quaternary ammonium salts as alkenyl sources. Substituting gaseous precursors for solid ones, not onlyleads to simpler experimental setups but improves practicability and applicability of those reactions.



For gaseous olefins e.g. ethenyl, 1-propenyl, 1-butenyl:



POSTER #16

Name / affiliation: Viktor Savic / TU Wien, FG BSC, Institute of Applied Synthetic Chemistry

Title: Modified Phosphatidylinositols for the Investigation of Peptide Assemblies

Viktor Savic, Christian Stanetty and Marko Mihovilovic*,

BSC-Group, Institute of Applied Synthetic Chemistry, TU Wien, Getreidemarkt 9, 1060 Vienna

Phosphatidylinositolphosphates (PIPs) are phospholipids composed of a polar inositol headgroup connected to a diacylglycerol (DAG) via a phosphate ester. Playing a crucial role in signal transduction pathways, PIPs mainly serve the purpose of enabling Ca^{2+}-flux from the endoplasmatic reticulum throughenzymatic liberation of inositoltriphosphate (IP_3). In recent years, it was found that PIPs are also a criticalfactor for the stabilization of the serotonin transporter´s (SERT) oligomeric structure, determined via mutational studies and single molecule light microscopy.[1-2]

To enable in-depth elucidation of the interactions between PIP and SERT, the need for fluorescent and stable analogues of PIP has arisen. Therefore, the presented work will deal with the synthesis of such analogues, spanning from a simple model compound, as a proof-of-concept regarding the feasibility of chemical synthesis of such scaffolds, to structurally and functionally more complex and diverse membersof the PI-family. The structural modifications to the PIP-framework will lead to more stable, photo-linkableand fluorescent representatives of this class of natural compounds. This will be achieved through introduction of handles enabling “click-chemistry” (e.g. azides, alkynes) and thereby conjugation to fluorophores, as well as carbene donors (e.g. diazirines) for means of irreversible photo-linking to SERT on the site of the DAG. Furthermore, an in-depth look into inositol desymmetrization will be presented, aswell as methods for the regioselective synthesis of asymmetrical diacylglycerols.

1. Anderluh, A.; Sitte, H. H.; Schütz, G. J., Direct PIP 2 binding mediates stable oligomer formation of theserotonin transporter. *Nature communications* **2017,** *8*, 14089.
2. Buchmayer, F.; Sitte, H. H., Amphetamine actions at the serotonin transporter rely on the availability of phosphatidylinositol-4,5-bisphosphate. *Proceedings of the National Academy of Sciences* **2013,** *110* (28), 11642-11647.

POSTER #17

Name / affiliations: Dominik Schnalzer / TU Wien, FG BSC

Title: Development of Novel Pyrazoloquinolinone Ligands for Selective and Site-Specific Modulation of $GABA_A$ Receptors

Dominik SCHNALZER[a], Florian VOGEL[b], Jure FABJAN[b], Filip KONIUSZEWSKI[b], Benjamin SCHAAR[b], Michael SCHNÜRCH[a], Margot ERNST[b] & Marko D. MIHOVILOVIC[aa]Institute of Applied Synthetic Chemistry, TU Wien
[b]Department of Pathobiology of the Nervous System (Center for Brain Research), Medical University Vienna

$GABA_A$-receptors are among the major neurotransmitter receptors in the mammalian brain and are involved in conditions such as anxiety disorder, epilepsy, and insomnia. The ligand-gated ion channels are composed of five different subunits encoded by 19 different genes. This results in numerous potential subunit combinations, and hence, a highly complex pharmacology. $GABA_A$ receptors are prominent targets for many pharmacologically and clinically important drugs such as benzodiazepines, barbiturates, neuroactive steroids, anesthetics, and convulsants which bind to various binding sites.

Several pyrazoloquinolinone ligands (PQs) with high potency for $GABA_A$ receptors have been reported. These allosteric modulators potently act via a binding site at extracellular α+/β− interfaces. However, this chemotype also tends to interact promiscuously with other binding sites on the receptor, including the benzodiazepine binding site at α+/γ− interfaces and the etomidate binding site. To further complicate matters, previous research has demonstrated that even minor changes in the pharmacophore features of PQs can lead to radical changes in ligand binding properties.

The presented work shows the design and synthesis of novel PQ-ligands that aim to address persistent challenges such as binding site selectivity and receptor subtype specificity. Several compounds within this series show high efficacy while concurrently showcasing the intricate nature of the structure-activity landscape. This work also unveiled a highly promising compound, which exhibits extremely strong modulation of GABA-induced currents. Presently, the pharmacological profile of this compound is being studied through *in vivo* experiments.

Furthermore, isotope-labeled PQs have been developed to unravel the complex structure-activity relationships and decipher the complex code of $GABA_A$ receptor pharmacology.

Name /affiliation: Lena Schwarz, Institute of Science and Technology Austria (ISTA)

Title: Hidden targets of autism spectrum disorders: dissecting divergent and convergentcausal paths

Abstract

Autism spectrum disorders (ASD) are associated with a high genetic heterogeneity, yet for the majority of the cases the underlying molecular causes remain elusive. Thus, it is important to identify molecular mechanisms responsible aiming to identify points of convergence and divergence. Here, we are aimingto identify commonly dysregulated regulatory elements throughout neurodevelopment, by making use of single-cell multi-omic technologies, which enables single-nucleus RNA-sequencing (snRNA-seq) coupled with single-nucleus ATAC-sequencing (snATAC-seq) of brains from various mouse models at multiple developmental stages.

We profiled ~200.000 cells obtained from the cerebral cortex of female and male mice at three different developmental stages (embryonic day (E)14.5, postnatal day (P) 4 and P14) from 11 ASD-risk genes. Interestingly, we found that all genotypes converge on a subset of glial cells throughout neurodevelopment showing a reduced cellular abundance, indicative of a global developmental delay. During the prenatal period, especially at P4, multiple cortical regulation and refinement processes are taking place. Here, several ASD-risk genes share a set of differently expressed genes, intersecting ona subclass of dysregulated mechanisms.

In summary, our data suggests neurodevelopmental abnormalities specific to certain cell types that are commonly affected among ASD-risk genes, therefore discovering convergent and divergent causal paths in the neurobiological foundation of how distinct risk genes contribute to ASD pathology.

Name / affiliation: Nadja K. Singer / University of Vienna, Institute of Theoretical Chemistry

Title: Investigating Photoswitchable Drugs: The Effect of *para*-Substitution on the Thermal Z/E Isomerization Mechanism of Arylazo-pyrazoles

Abstract:

Nadja K. Singer[a,b], Katharina Schlögl[c], Patrick Zobel[a], Marko D. Mihovilovic[c], Leticia González[a];

[a] Institute of Theoretical Chemistry, University of Vienna, Austria; [b] Vienna Doctoral School in Chemistry (DoSChem), University of Vienna, Austria; [c] Institute of Applied Synthetic Chemistry, TU Wien, Austria

Arylazo-pyrazole photoswitches are molecules able to efficiently interconvert between two photoisomers upon light irradiation, which makes them sought after for applications in photopharmacology. For their potential application as drugs, a clear understanding of the thermal isomerization mechanism to the stable isomer and the associated half-life is necessary. Here we present the computational unravelling of the controversial thermal Z/E-isomerization of the unsubstituted arylazo-pyrazole (more specifically: phenylazo-1,3,5-trimethylpyrazole), providing evidence for the involvement of a multistate rotational isomerization mechanism, and we calculate the overall thermal half-life [1]. There are multiple different thermal isomerization mechanisms known for azobenzene derivatives [2]. Most notably: (i+ii) the in-planeinversions of one or the other phenyl (i) / heteroaryl (ii) moiety around its neighboring azo nitrogen and

(iii) the out-of-plane rotational mechanism around the azo-bond. However, for azobenzene a fourth mechanism (iv) involving intersystem crossing from S_0 to T_1 and back to S_0 can occur in a mechanism similar to the rotational transition mechanism [2]. The associated half-lives of mechanisms (i-iii) and (iv) can be calculated using conventional and non-adiabatic transition state theory, respectively. Furthermore, we use the obtained in-depth knowledge from the investigation of the unsubstituted phenylazo-1,3,5-trimethylpyrazole and transfer it to 8 *para*-substituted compounds.

We show that, while the unsubstituted compound is driven by a mixture of mechanisms (i) and (iv), *para*-substitution influences this composition of mechanisms. For electron donating groups we see an increased importance of mechanism (iv), whereas for electron withdrawing groups the same holds true for mechanism (i). In summary, we can reproduce experimental half-lives and explain their *para*-substitution trends with a change in the operating thermal Z/E-isomerization mechanisms.

References: *[1] N. K. Singer, K. Schlögl, P. Zobel, M. D. Mihovilovic, L. González: Singlet and Triplet Pathways Determine the Thermal Z/E Isomerization of an Arylazopyrazole-Based Photoswitch (2023), submitted.; [2] Axelrod S., Shakhnovich E., Gómez-Bombarelli R., ACS Cent. Sci. (2023) 9, 166–176.*

POSTER #20

Name / affiliation: Aljoša Smajić, University of Vienna, Department of Pharmaceutical Sciences

Title: Identifying Differences in the Performance of Machine Learning Models for off-Targets trained on publicly available and proprietary datasets

Aljoša Smajić, Iris Rami, Sergey Sosnin, Gerhard F. Ecker University of Vienna, Department of Pharmaceutical Sciences

Established publicly available databases such as ChEMBL allow researchers to use information without constrictions and create predictive tools for a broad spectrum of applications in the field of toxicology. However, large amount of these entries origin from positive experimental results as they are more likely to be published. When observing the tactics employed by pharmaceutical companies regarding testing molecules, it becomes evident that a significant number of generated results are negative. Therefore, we investigated the distribution of positive and non-positive entries within ChEMBL for a set of off-targets and its impact on the performance of classification models when applied to pharmaceutical industry datasets. Results show that models trained on publicly available data tend to overpredict positives, and models based on industry data show the opposite. Visualization of the prediction space for a set of 10000 compounds further strengthens this and allows to identify areas in the chemical space where predictions converge. Finally, we highlight the utilization of these models for consensus modeling for potential adverse events prediction.

POSTER #21

Name / affiliation: Maximilian Xaver Tiefenbacher, University of Vienna, Institute of Theoretical Chemistry, González research group

Title: Estimating thermal isomerization half-lives of arylazopyrazole photoswitches using neural network potentials

Abstract:

Co-authors: Johannes Dietschreit[a], Simon Axelrod[a], Rafael Gómez-Bombarelli[a], Leticia González[b]
a: Massachusetts Institute of Technology
b: University of Vienna

Photoswitches are a class of molecules with the ability to change between two distinct configurations by absorbing light. This property gives them a broad range of applications such as energy storage, nanomachines, or photodrugs. Photodrugs are typically inactive molecules when administered to the patient that can be activated by light in a specific region. An important property of photoswitchable drugs is the rate at which they thermally switch, i.e., the rate of change from the energetically less favorable conformation to the more stable one. This thermal isomerization can span from a few milliseconds to years, and thus, the possible application of a specific photoswitch is conditioned by its thermal rate.

In this contribution, we focus on arylazopyrazole photoswitches. Our aim is to understand how chemical alterations change the thermal switching rate between the cis and trans isomers. Previous research on the reaction rate of this compound group [1] investigated the thermal isomerization mechanism of the parent compound and a few derivatives by means of quantum chemical methods and experiments. Here we use machine learning to explore approximately 31 000 derivatives and estimate their thermal isomerization half-lives. To this aim, we used the well-established neural network PaiNN [2] to predict the potential energy surfaces necessary to investigate thermal relaxation. We obtained the training data for those potentials with time dependent density functional theory calculations. Because of the chemical similarity between the compounds, we were able to reduce the amount of data points needed for the training set by a large fraction. This allowed us to obtain a neural network capable of predicting the energetic states of those molecules. The neural network is able to interpolate between similar chemical species and understand relevant information that they share. Therefore, only a subset of all investigated molecules is sufficient as a basis for the training set. This is relevant since the number of molecules makes it impossible to obtain a large amount of data from every single molecule. In addition, we relied on a process called active learning [3], which enables the neural network to decide which data points it needs to better predict the barrier between the different configurations. This process promises a faster way to predict reaction rates of arylazopyrazoles.

[1] N.K. Singer, K. Schloegl, J. P. Zobel, M. Mihovilovic and L. González, Singlet and Triplet Pathways Determine the Thermal Z/E Isomerization of an Arylazopyrazole Photoswitch, [submitted]
[2] K. Schütt, O. Unke and M. Gastegger, Equivariant message passing for the prediction of tensorial properties and molecular spectra. Intern. Conference on Machine Learning 2021, 139, 9377-9388
[3] *S. Axelrod, E. Shakhnovich, and R. Gómez-Bombarelli, ACS Central Science, 2023, 9 (2), 166-176*

POSTER #22

Name / affiliations: Florian D. VOGEL / Medical University of Vienna, Department of Pathobiology of the Nervous System (Center for Brain Research)

Title: GABRA4 variants contribute to neurodevelopmental abnormalities with or without seizures

Abstract

$GABA_A$ receptors are a protein family highly expressed in many tissues, mostly in the central nervous system. They regulate excitation/inhibition balance (EIB) in the brain, and act as a modulating system. Disruption of the EIB can lead to severe diseases like seizure / epileptic disorders. Upon binding of the (neuro-)transmitter gamma amino butyric acid (GABA) they transition into an open state at which they conduct chloride and to a lesser extent bicarbonateions, mainly causing hyperpolarization in $GABA_A$ receptor expressing cells. Many of the 19 human paralog GABAA receptor genes are associated with seizure disorders, including the heavily studied α1, β2/3 and γ2 subunits.

In the past, GABAA receptors in epilepsy were mainly studied in the context of receptors contributing to phasic inhibition. Those reside in the postsynaptic cell membrane and share rapid kinetic properties which allow fast communication between two cells. Previously, we reported a patient with a mutation in *GABRA4*, encoding for the α4 subunit of $GABA_A$ receptors, as the first evidence for an association between *GABRA4* and neurodevelopmentaldelay with seizure disorder. The α4 subunit is a major contributor to extrasynaptic receptors providing so called “tonic inhibition”, which is elicited by very low ambient or spill-over GABAconcentrations and causes a constant flow of ions, maintaining the EIB. Electrophysiological recordings in *Xenopus laevis* oocytes unraveled accelerated desensitization and a loss of seizure protective neuroster oid effect as main characteristics of receptors harboring the a4T300I mutation.

Since our first work was published, three additional patients carrying *GABRA4* mutations andin part overlapping phenotypes were identified. We utilize the power of molecular dynamics simulation with the aim to understand how *GABRA4* variants change receptor function and thus contribute to seizure disorders. With this, we establish *GABRA4* as a risk gene for neurodevelopmental delay with or withoutseizures. Our collective efforts will help to identifyaffected individuals in the future by encouraging screening for this specific gene in standard screens. Additionally, our pharmacological findings hopefully shape future studies to providepersonalized therapies to individual patients and *GABRA4* variants.

POSTER #23

Name / affiliation: Yi Xiao, Institute of Organic Chemistry, University of Vienna

Title: Development of Novel Fluorescent Dyes and Their Application as Probes to Study BiologicalProcesses

Abstract: *Yi Xiao[1], Oliver Belleza[2], Sergio Armentia Matheu[1], Nina Kastner[2], Iakovos Saridakis[1], Stefanie Rukavina[1], Margaux Riomet[1], Harald Sitte[2], Nuno Maulide[1]*

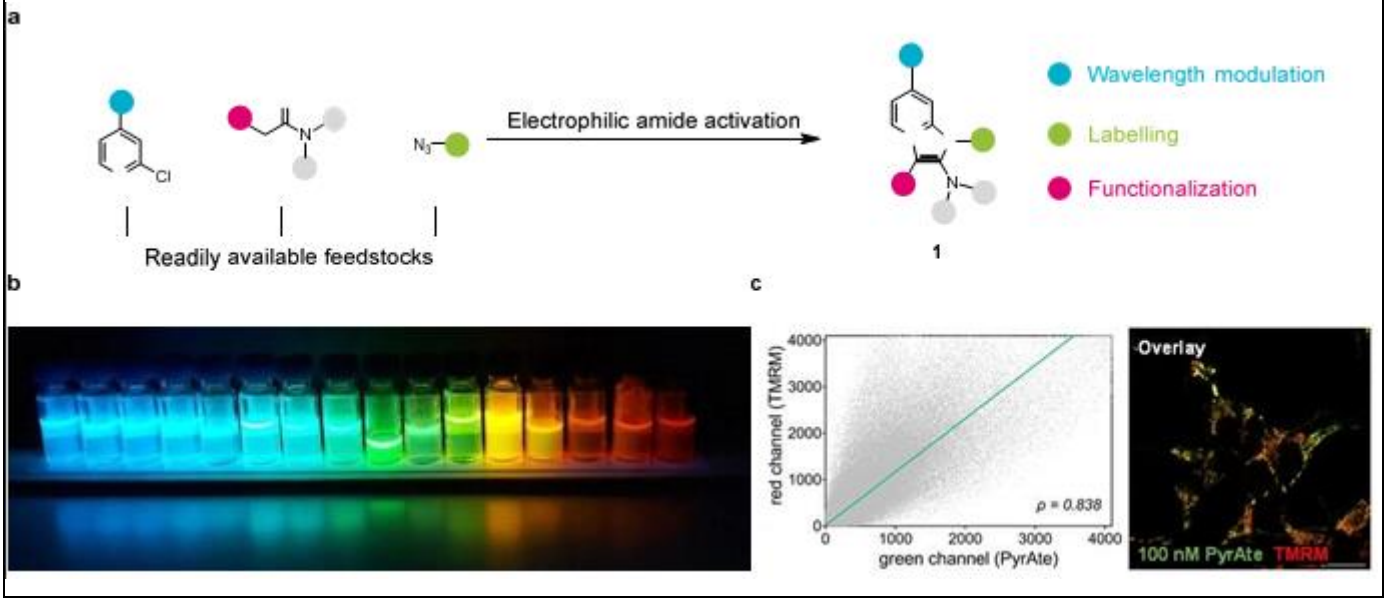
1 Faculty of Chemistry, Institute of Organic Chemistry, University of Vienna

2 Center for Physiology and Pharmacology, Institute of Pharmacology, Medical University of Vienna

Used as probes for the detection, visualization and characterization of biological molecules, synthetic fluorescent compounds have become indispensable tools in chemical biology. Over the last years, diverse families of fluorescent compounds, such as coumarins, BODIPYs and rhodamines, have risen to prominence and are widely employed in the field. In searching for even more powerful dyes, efforts have focused on increasing the quantum yield, making the compounds more (photo)stable and increasing the Stokes shift.

Our group's continued investigations of the reactivity and synthetic utility of keteniminium species, derived from electrophilic amide activation, has resulted in a wide range of applications. In 2016, we reported the use of azides to transform keteniminium species into α-aminated amides. Interestingly, an unexpected reaction of azides with benzylic amides led to the serendipitous discovery of highly fluorescent imidazopyridinium triflates, known as PyrAtes (**1**, Fig. a).

Herein, we present the use of a modular synthetic method, employing commercially available or easily accessible feedstock materials (Fig. a), to access a library of PyrAtes. In thorough investigations, we have established the effects of substituent variation and have found that careful modulation of the chemical structure allows fine-tuning of the emitting wavelengths (Fig. b). PyrAtes have been found to be actively sequestered by mitochondria, allowing them to be used as novel mitochondria markers (Fig. c).



POSTER #24

Name / affiliation: Haoqi Zhang / University of Vienna, Institute of Organic Chemistry / Christian-DopplerLaboratory for Entropy-Oriented Drug Design

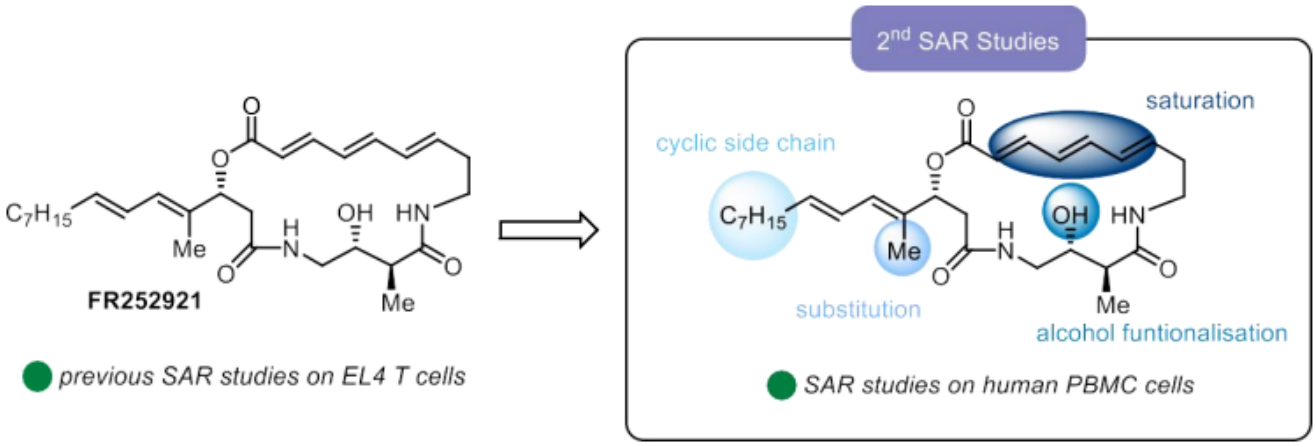
Title: Synthesis of FR Analogues and its Application in Human PBMCs

Haoqi Zhang,[a,b] Manuel Schupp,[a] Iakovos Saridakis,[a] Thomas Leischner,[a] Nuno Maulide[a,ba]*

University of Vienna, Institute of Organic Chemistry

[b] Christian-Doppler Laboratory for Entropy-Oriented Drug Design

Immunosuppressive agents are essential for the modern treatment of autoimmune diseases and suppressionof allograft rejection. However, all clinically approved immunosuppressants are commonly associated withside effects and limitations.[1] The macrocycle FR252921 is a natural product whose immunosuppressive properties have yet to be fully understood. Our group has achieved a convergent total synthesis of FR252921 including 11 additional analogues and were tested on EL4-T-lymphocyte cells[2]. In order to further investigate/study the structure-activity relationship, we have designed 13 additional analogues, including various modifications on the macrocyclic core that was not explored previously. These analogues were evaluated on human PBMC cells, focusing on their toxicity and immunosuppressive activities, including T-cell and NK-cell inhibition.



[1] B. D. Kahan, *Nat. Rev. Immunol.* **2003**, *3*, 831–838.
[2] Y. Chen, G. Coussanes, C. Souris, P. Aillard, D. Kaldre, K. Runggatscher, S. Kubicek, G. Di Mauro, B.Maryasin, N. Maulide, *J. Am. Chem. Soc.* **2019**, *141*, 13772–13777.

Name / affiliation: Katharina Schlögl / TU Wien, Institute of Applied Synthetic Chemistry

Title: Photoswitchable Tool Compounds for the Reversible Stabilization of G-Quadruplex DNA

Katharina Schlögl[1], Davia Prischich[2], Marko D. Mihovilovic[1], Matthew J. Fuchter[2]
[1]Institute of Applied Synthetic Chemistry, TU Wien, Austria
[2]Department of Chemistry, Imperial College London, United Kingdom

G-quadruplexes (G4s) are secondary DNA structures formed in guanine-rich DNA sequences and are believed to influence genome functions like transcription, genome stability and many more. Often, an increase of G4s is detected in precancerous cells compared to respective parent healthy cells, which renders G4s an emerging research topic in the field of cancer biology for therapeutic targeting. Researchers use G4 stabilizing ligands like Pyridostatin to force the formation of the G4 structure to make them more accessible for investigations.

Photopharmacology is a research field connecting pharmacology and photochemistry of bioactive compounds. By incorporating photoswitchable moieties, known as photoswitches, into pharmacologicallyactive agents, photopharmacology enables light-induced, highly precise temporal and spatial control of several biological targets, including DNA. Such photoswitches are able to change between two conformations by irradiation with light.

To harness this advanced control over biological targets via photopharmacology, we synthesized and investigated several heteroaromatic photoswitches as potential G4 stabilizers. Such photoswitchable G4 stabilizing compounds could be used to reversibly induce folding and unwinding of the respective G4s. Multiple benzimidazole- and quinoline-based photoswitches were prepared and photophysically characterized. These investigations paved the way for the development of a promising photoswitchable azopyrazole-Pyridostatin analogue (Figure 1), whose biological evaluation is currently pending.

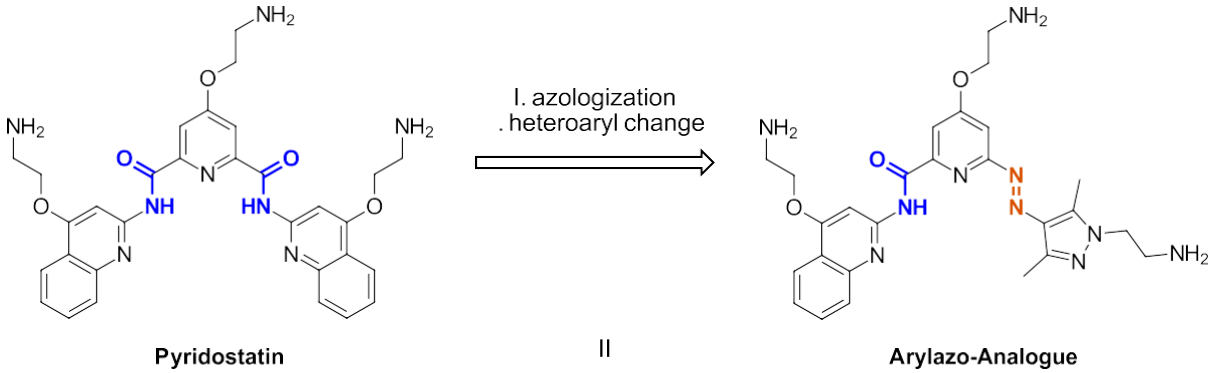


Figure 1: Design pathway from known G4 stabilizer Pyridostatin towards a photoswitchable arylazo-analogue.

POSTER #26

Name: Marco Niello[a,1,§] , Spyridon Sideromenos[b] , Ralph Gradisch[a] , Ronan O´Shea[c], Jakob Schwazer[a], Julian Maier[a], Nina Kastner[a] , Walter Sandtner[a], Kathrin Jäntsch[a], Carl R. Lupica[c] , Alexander F. Hoffman[c] , Gert Lubec[d],Claus J. Loland[e] , Thomas Stockner[a] , Daniela D. Pollak[b], Michael H. Baumann[f] , and Harald H. Sitte[a,g,1]

Author affiliations:

[a,b,g] Medical University of Vienna, 1090 Vienna, Austria;
[c] Electrophysiology Research Section, National Institute on Drug Abuse, NIH, Baltimore, USA
[d] Paracelsus Medical University, 5020 Salzburg, Austria;
[e]University of Copenhagen, Denmark;
[f] National Institute on Drug Abuse, NIH, Baltimore, USA
[§] **Current affiliation: Genetics of Cognition Laboratory, Neuroscience Area, Istituto Italiano di Tecnologia**
[1]***To whom correspondence may be addressed.*** Email: marco.niello@iit.it

Title: Persistent binding at dopamine transporters determines sustained psychostimulant effects

Abstract In the human body, the interaction between a drug and its target is influenced by the constant flux of fluidsand a variety of physiological processes. However, the pharmacology of different compounds is classicallyassessed in vitro under thermodynamic equilibrium, which is far from the dynamic conditions in vivo. Consequently, the in vivo properties of a drug often differ from the in vitro thermodynamic equilibrium. Psychostimulants interact with the dopamine transporter (DAT) and are used for the treatment of differentneuropsychiatric disorders. However, they also produce substance abuse disorders and often the in vitro pharmacology, assessed under thermodynamic equilibrium, does not fully justify their different pharmacological profile in vivo. We used radiotracer assays, computational approaches, transporter electrophysiology and behavioural assays in mice to investigate the role of binding kinetics in the psychomotor effects elicited by different psychostimulants. We found that binding kinetics of different psychostimulants and DAT-inhibitors correlates with the duration of the psychomotor effect in vivo. Subtle modifications of their chemical structure produced enantioselective effects in their in vitro and consequently in vivo pharmacology. Moreover, high affinity psychostimulants tested, showed similar slow dissociation rates leading to a pseudo-irreversible binding kinetics at DAT. The pseudo-irreversible binding kinetics is responsible for the observed non-competitive pharmacology, correlates with persistent psychostimulant effects in mice and differs from the fast-acting DAT-inhibitor cocaine. Our work shows that drug binding kinetics at DAT can have a significant impact on the psychostimulant effects of drugs in vivo. Moreover, it also shows that for their acute effects binding kinetics has a primary importance compared to metabolic routes. Our study might provide insights for the design of novel DAT inhibitors with improved clinical utility and it highlights that research on illicit drugs may help to reach a better understanding of physiological and toxicological processes.

Acknowledgements: The authors a acknowledge the NIDA drug supply program for supplying α-PVP enantiomers.
Keywords: psychostimulants; dopamine transporter; binding kinetics; cathinones

POSTER #27

Name / affiliation: Eva Maria Plessl, Division of Pharmacology and Toxicology, University of Vienna

Title: PeptAIDes – how I got a PostDoc grant

Abstract:

If you want to stay in science and are looking for an opportunity for a PostDoc position, you will hear the following sentence repeatedly: “Do you have your own funding?” As a young scientist, there is hardly anything more intimidating than this phrase. Getting funding is challenging and sometimes can feelimpossible. I am here to tell you I felt (and still feel) the same way.

As a group of five young PostDocs, we applied for a unique postdoctoral program for innovative interdisciplinary teams, the so-called “Zukunftskolleg”, funded by the FWF. More than 50 groups applied, five were invited for an interview, and four groups got funded. We were one of them. Together we got funding for 2 PostDoc positions, 5 Ph.D. students, one technician, material and travel costs for 4 years total, which sums up to >1.7M €.

I would be happy to share with you what we did to achieve this at my poster.Heads up – you will need to be both, resilient and lucky.