"Adhesion GPCRs - present and future"

October 3rd-5th 2023

Book of Abstracts

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Speakers

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Jeff Savas	(Northwestern University, USA)
Xianhua Piao	UCSF, USA)
Patrick Barth	EPFL, Switzerland)
Anne Bormann	(Leipzig University, Germany)
Peter Hildebrand	(Leipzig University, Germany)
Florian Seufert	(Leipzig University, Germany)
Sandra Berndt	(Leipzig University, Germany)
Stephanie Pick	(HHU Düsseldorf, Institute of Cell Biology, Germany)
Sandra Žáčková	(Institute of Organic Chemistry and Biochemistry of Czech Academy of Sciences, Czech Republic)

Programme

October 3rd (Tuesday) 2023

08:00 – 09:00	Arrival & Registration for MC members
09:00 – 12:00	***MC meeting*** (Coffee served during the meeting))
12:00 – 13:30	Lunch at MedILS
13:30 – 15.30	***Workgroup meetings***
15:30 – 16:00	Coffee break, registration continued
16:00 – 17:40	**Session #1 (Chair Ines Liebscher, Leipzig University, Germany)**
16:00 – 16:05	Opening word (Katarina Trajkovic, MedILS)
16:05 – 17:00	**Xianhua Piao (virtual)***University of California, USA*
	ADGRG1/GPR56 Regulates Microglial Actin Dynamics and Phagocytosis to Limit Alzheimer's Disease Progression
17:00 – 17:40	**Anne Bormann**
	Leipzig University, Germany
	Intron retention of an adhesion GPCR generates single transmembrane-helix isoforms to enable 7TM-adhesion GPCR
	function
17:40 – 19:00	***Posters***
19:00 – 20:30	Dinner at MedILS
20:00 - 23:00	Conference Banquet

October 4th (Wednesday) 2023

09:00 - 12:40	**Session #2 (Chair Sasa Jovanovic, Leipzig University, Germany)**
09:00 - 10:00	**Patrick Barth**
EPFL, Switzerland	**Uncovering and engineering the mechanical properties of the adhesion GPCR ADGRG1 GAIN domain**
10:00 - 10:40	**Peter Hildebrand**
	Leipzig University, Germany
	Mechanistic insights into receptor mediated G protein activation
10:40 - 11:10	**Posters** / coffee break
11:10 - 11:50	**Florian Seufert**
	Leipzig University, Germany
	Unveiling the GPS Cleavage Mechanism in ADGRL1 with QM/MM
11:50 – 12:40	Tour around MedILS (Ana-Marija Vuckovic)
12:40 – 14:10	Lunch at MedILS
14:10 – 16:30	**Session #3 (Chair Katarina Trajkovic, MedILS)**
14:10 - 15:10	**Jeff Savas**
	Northwestern University, USA
	Identification of ligand-receptor interactions using extracellular domain Fc fusion protein baits and shotgun proteomic analysis
15:10 – 15:50	**Sandra Berndt**
	Leipzig University, Germany
	GPR126/ADGRG6 Arrestin Interaction
15:50 – 16:30	Coffee break
17:30 – 19:30	Guided city tour for interested participants
19:30 – 21:00	Free activities / dinner in the city (Bistro Ka’ doma) for interested participants

October 5th (Thursday) 2023

09:00 – 11:20	**Session #4 (Chair Simone Prömel, Heinrich Heine University Düsseldorf, Germany)**
09:00 - 10:00	**Nicole Scholz**
	Leipzig University, Germany
	Adhesion GPCR-dependent communication across cells
10:00 – 10:40	**Stephanie Pick**
	HHU Düsseldorf, Institute of Cell Biology, Germany
	The adhesion GPCR ADGRL1/LPHN1 is involved in the regulation of food intake
10:40 - 11:20	**Sandra Žáčková**
	Institute of Organic Chemistry and Biochemistry of Czech Academy of Sciences, Czech Republic
	The expression changes of selected adhesion G-protein-coupled receptors in lung adenocarcinoma and colorectal
	carcinoma cell lines infected with SARS-CoV-2 and the effect of their knock-down on viral replication
11:30 - 13:00	Lunch at MedILS
13:00	Departure

Lectures

Intron retention of an adhesion GPCR generates single transmembrane-helix isoforms to enable 7TM-adhesion GPCR function

Bormann, A[1], Körner, MB[1,2], Dahse, A-K[1], Gläser M-S[1], Lede, V[3], Schöneberg, T[3], Bigl, M[1], Langenhan, T[1], Ljaschenko, D[1]✉, Scholz, N[1]✉*

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In Drosophila, mechanosensation in sensory neurons is modulated by the adhesion GPCR ADGRL/Latrophilin/Cirl. Cirl is encoded by a single gene and alternative splicing was predicted to produce transcripts encoding structurally distinct proteins containing either seven transmembrane helices (Cirl^{7TM}), hence a classical GPCR layout and proteins containing only a single TM (Cirl^{1TM}) that lack the classical signaling unit. However, whether such transcripts are translated into proteins and what functions they serve in vivo is unknown.

In this study, we engineered a transgenic toolkit to express differently modified Cirl proteins, i.e. Cirl isoforms, under the transcriptional control of its endogenous promoter, and we could show that Cirl^{1TM} protein is indeed produced in vivo. It is expressed in central and peripheral nervous tissues, including mechanosensory neurons. Biochemical analysis and functional recordings showed that co-expression of both, Cirl^{7TM} or Cirl^{1TM}, isoforms is required for normal mechanosensory neuron function. Cirl affects the cAMP levels in those neurons likely through $G\alpha_o$. Co-immunoprecipitation experiments suggest a direct protein interaction between Cirl^{7TM} and Cirl^{1TM}, which appears to involve the N terminal fragment (NTF) of Cirl^{1TM}. Importantly, co-expression of membrane attached Cirl^{1TM}-NTF and the full length Cirl^{7TM} sufficed to rescue the mechanosensitive deficit of Cirl^{KO} mutants. Collectively, our data suggest a model in which the Cirl^{1TM}-NTF can act as a ligand for Cirl^{7TM}, a mode of action required for normal mechanosensation of these sensory neurons.

Unveiling the GPS Cleavage Mechanism in ADGRL1 with QM/MM

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Adhesion G-protein coupled receptors (aGPCR) are a family of 32 mammalian proteins with a defining conserved GPCR autoproteolysis inducing (GAIN) domain that catalyzes receptor self-cleavage at a GPCR proteolysis site (GPS). The autoproteolytic mechanism has been previously proposed, but remains to be validated since.[1,2]

A previous computational study has uncovered variable flexible protein regions, whose dynamics mediate solvent-accessibility of the catalytically active GPS triad HL|S/T, however classical molecular dynamics approaches fall short of explaning the chemical reaction.[3]

Using a multiscale QM/MM approach - combining computational quantum mechanics with classical molecular dynamics - to study the GAIN domain cleavage mechanism *in silico* on ADGRL1 reveals the likely sequence of events at the electronic level, suggesting relative energies for the individual states during the reaction, and provides insight into the structural determinants for a successful GPS cleavage exceeding the catalytically active GPS triad.

The most likely cleavage pathway can be uncovered by directly scanning and comparing sequences of reaction steps, and the individual contribution of surrounding protein residues can be elucidated. A stable π-edge contact with a conserved phenylalanine provides an interaction which is crucial to generate the initial reaction step, where the His^{-1} deprotonates the Thr^{+1} hydroxyl moiety for nucleophilic attack on the peptide carbon, forming a hydroxy-oxazolidine intermediate. Biochemical experiments on the importance of this residue showed that restoring the Phe-His interaction in the uncleaving ADGRB3 GAIN domain partially re-instates cleavage, while its deletion reduces cleavage in the ADGRL1 GAIN domain.[4] By investigating the pathway from different angles, we further find surprising involvement of an acid/base catalysis mediated via water molecules by a glutamate residue with shifted pKa.

The residues involved in GAIN autoproteolysis include conserved positions, which are revealed by a bioinformatics approach using AlphaFold models of all aGPCR GAIN domains. This approach establishes a Generic Residue Numbering Scheme which can structurally map all GAIN domains onto one another.

Identification of ligand-receptor interactions using extracellular domain Fc fusion protein baits and shotgun proteomic analysis

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Ligand-receptor interactions facilitate signaling between cells and cells and the extracellular space. Despite their importance, the identification of ligand-receptor interactions remains challenging due to technical barriers. We have developed a discovery-based analysis workflow that couples affinity purification with mass spectrometry (MS) and bioinformatic analysis. Compared to previous approaches, our approach increases sensitivity, shortens analysis time, and enhances comprehensiveness. Receptor extracellular domains are fused with the Fc region of IgG that are purified from transfected HEK293 cells. These 'ecto-Fcs' are coupled to Protein A beads and serve as bait for binding assays with prey proteins extracted from brain. After capture, the affinity-purified proteins are digested into peptides and comprehensively analyzed by MS (ecto-Fc MS). Ecto-Fc MS outperforms antibody-based approaches and provides a robust framework for identifying extracellular ligand-receptor interactions.

We have used ecto-Fc MS to identify seven ligand-receptor interactions including three adhesion G protein-coupled receptors (AGPCRs). First, we focused on Latrophilins (LPHNs), a small family of AGPCRs that mediate the colossal synaptic vesicle exocytosis caused by the black widow spider venom a-latrotoxin. We identified Flrt and Ten-m / Odz proteins as postsynaptic LPHN ligands. We reported that the ectodomains interact in trans with high affinity and that this interaction regulates excitatory synapse density in cultured neurons. We also found that, reducing FLRT3 levels in vivo decreases afferent input strength and dendritic spine number. Second, we identified the orphan receptor GPR158 as a binding partner for the heparan sulfate proteoglycan glypican 4 (GPC4). GPC4 is enriched on hippocampal mossy fibers, whereas postsynaptic GPR158 is restricted to the proximal segment of CA3 dendrites. GPR158 induced presynaptic differentiation in contacting axons and this effect required GPC4 and LAR. Loss of GPR158 increased mossy fiber synapse density but disrupts synaptic organization and reduced synaptic strength. Taken all together ecto-Fc MS accelerates the characterization of AGPCRs.

Adhesion GPCR-dependent communication across cells

Nicole Scholz[1,3], Anne-Kristin Dahse[1,3], Marguerite Kemkemer[1], Anne Bormann[1], Genevieve M. Auger[1], Lucia F. Ernst[1], Max Buhlan[1], Amelie Meyer-Mölck[1], Yin Kwan Chung[1], Franziska Klose[2], Mohamed Ali Jarboui[2], Tobias Langenhan[1]

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Presenting and corresponding Author: Nicole Scholz

Adhesion GPCR (aGPCR) are expressed in every organ system of the human body. They represent a large and rather peculiar group of cell surface receptors that combine mechanosensitivity, tethered agonism and autoproteolysis otherwise known from integrins, protease-activated receptors and polycystines, respectively.
aGPCR autoproteolysis is mediated by its evolutionarily conserved GPCR autoproteolysis-inducing (GAIN) domain. The resulting N- and C-terminal fragments (NTF, CTF) remain non-covalently linked to form heterodimeric receptor molecules. Separation of NTF-CTF heterodimers followed by exposure of the tethered agonist (*Stachel*) is one of the prevailing concepts of how aGPCR are activated. Yet, whether receptor activating NTF-CTF dissociation is physiologically relevant remains controversial.
Here, I will introduce a novel transgenic sensor system designed to track NTF-CTF separation of aGPCR and will present new insight to latrophilin-type aGPCR Cirl/ADGRL receptor function in neural processes *in vivo* in *Drosophila melanogaster.*

Uncovering and engineering the mechanical properties of the adhesion GPCR ADGRG1 GAIN domain

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Key cellular functions depend on the transduction of extracellular mechanical signals by specialized membrane receptors including adhesion G-protein coupled receptors (aGPCRs). While recently solved structures support aGPCR activation through shedding of the extracellular GAIN domain, the molecular mechanisms underpinning receptor mechanosensing remain poorly understood. When probed using single-molecule atomic force spectroscopy and molecular simulations, ADGRG1 GAIN dissociated from its tethered agonist at forces significantly higher than other reported signaling mechanoreceptors. Strong mechanical resistance was achieved through specific structural deformations and force propagation pathways under mechanical load. ADGRG1 GAIN variants computationally designed to lock the alpha and beta subdomains and rewire mechanically-induced structural deformations were found to modulate the GPS-Stachel rupture forces. Our study provides unprecedented insights into the molecular underpinnings of GAIN mechanical stability and paves the way for engineering mechanosensors, better understanding aGPCR function, and informing drug-discovery efforts targeting this important receptor class.

Mechanistic insights into receptor mediated G protein activation

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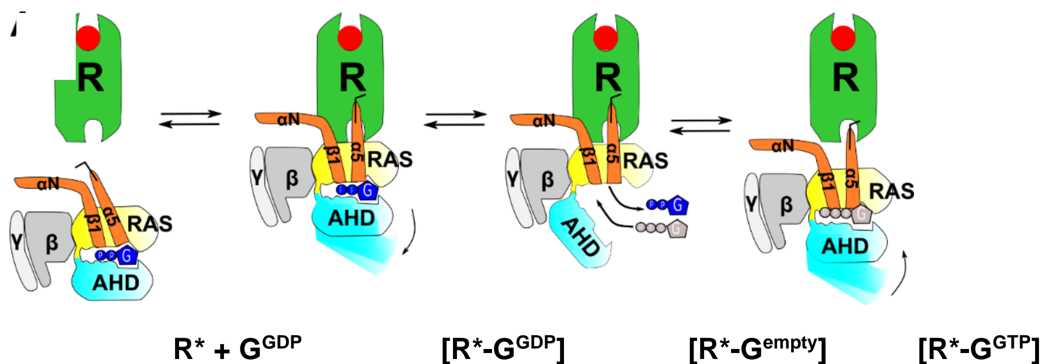

Heterotrimeric G proteins are activated by G protein-coupled receptors (GPCRs) that mediate the exchange of guanine nucleotide in the Gα-subunit. We are investigating the mechanism of receptor-mediated G protein activation to obtain insights into the sequence of events that govern this important process. This process is divided into (i) functional association of a G protein with the receptor, (ii) nucleotide exchange, and (iii) G protein activation. To obtain an atomic-level description of the sequence of these events, we combine classical µ-second molecular dynamics simulations with time-resolved cryo-EM[1]. Simulation of the association of the G protein with the β2-adrenoceptor (starting from the respective crystal structures[2,3]) describes the events that eventually lead to the ejection of GDP from the Galpha subunit, the rate-limiting step during G protein activation[4,5,6]. Transmission of the signal to the G protein occurs via a novel receptor interface confirmed by site-directed mutagenesis and functional assays. From this β2AR-Gs^{GDP} intermediate, the G protein must undergo an in-plane rotation against the receptor to reach the β2AR-Gs^{EMPTY} state. Combining time-resolved cryo-EM with molecular dynamics simulations reveals that such rotation in the opposite direction occurs during the final steps of Gs protein activation[1]. Thus, a corkscrew binding and unbinding pattern appears to underline the nucleotide exchange of G proteins by GPCRs. Our analysis sheds new light on the steps of receptor-mediated G protein activation and extends the limited view of nucleotide-free snapshots to include additional states and structural features responsible for signal transduction and specificity[6] of G protein coupling.

1. M. Papasergi-Scott *et al.* Time-resolved cryo-EM of G protein activation by a GPCR. *bioRxiv* 2023.03.20.53387 (2023)
2. Rasmussen, S. G. F. *et al.* Crystal structure of the β 2 adrenergic receptor-Gs protein complex. *Nature* (2011)
3. Liu, X. *et al.* Structural Insights into the Process of GPCR-G Protein Complex Formation. *Cell* (2019)
4. Landis, C. A. *et al.* GTPase inhibiting mutations activate the α chain of G s and stimulate adenylyl cyclase in human pituitary tumours. *Nature* (1989)
5. Scheerer, P. et al. Structural and kinetic modeling of an activating helix switch in the rhodopsin-transducin interface. Proc. Natl. Acad. Sci. (2009)
6. Du, Y. *et al.* Assembly of a GPCR-G Protein Complex. *Cell* (2019)
7. Rose, A. S. *et al.* Position of transmembrane helix 6 determines receptor G protein coupling specificity. *J. Am. Chem. Soc.* (2014)

GPR126/ADGRG6 Arrestin Interaction (lecture and poster)

Lisa Kupsch, Lusia Riedel, Ines Liebscher, Sandra Berndt

1 Mitgau J, Franke J, Schinner C, Stephan G, Berndt S, Placantonakis DG, Kalwa H, Spindler V, Wilde C, Liebscher I. The N Terminus of Adhesion G Protein-Coupled Receptor GPR126/ADGRG6 as Allosteric Force Integrator. Front Cell Dev Biol. 2022 Jun 23;10:873278. (doi: 10.3389/fcell.2022.873278)
2 Wilde C, Chaudhry PM, Luo R, Simon KU, Piao X, Liebscher I. Collagen VI Is a Gi-Biased Ligand of the Adhesion GPCR GPR126/ADGRG6. Cells. 2023 Jun 5;12(11):1551. (doi: 10.3390/cells12111551)
3 I KY, Huang YS, Hu CH, Tseng WY, Cheng CH, Stacey M, Gordon S, Chang GW, Lin HH. *Front Immunol.* 2017 Apr 3;8:373. (doi: 10.3389/fimmu.2017.00373)
4 Lee CS, Cho HJ, Lee JW, Son H, Chai J, Kim HS. *Stem Cell Reports*. 2021 Apr 13;16(4):868-882. (doi: 10.1016/j.stemcr.2021.03.003)

Adhesion GPCR, GRP126/ADGRG6 is activated by different extracellular matrix proteins, prion proteins or mechanical forces and mediates Gs or Gi signaling pathways [1,2]. Both pathways have different regulatory roles, like the differentiation of adipocytes, osteoblasts, and Schwann cells and on the other hand breast cancer proliferation. Next to classical G protein-mediated signaling cascades, GPCR stimulation can result in the activation of various mitogen activated protein kinases (MAPK). The impact of non-canonical signaling in these functions remains elusive. The activation of the downstream effectors like Src kinases and ERK1/2 has been reported for GPR126/ADGRG6 [3,4]. Nevertheless, the activation mechanism of the effectors as well as the functional role is not well understood. Often the activation occurs via scaffold proteins like arrestins. Here, we investigate GPR126/ADGRG6 arrestin interaction and its role in signaling bias. First, we showed the overall binding of arrestin to the receptor using Co-immunoprecipitation. We could detect a more robust binding of the CTF GPR126, which represents an activated form of the receptor. Next, we detected the receptor arrestin interaction using the NanoBiT complementation assay. Arrestins are known to bind to phosphorylated serine/threonine cluster. For the identification which phosphorylation pattern is present and crucial for arrestin binding, we are introducing systematic point mutations. GPR126/ADGRG6 has three potential phosphorylation cluster which we will analyze. In the field, it is discussed if the phosphorylation position is impacting the arrestin activation differently, which could cause a selectivity for specific effectors and therefore signaling cascades.

The expression changes of selected adhesion G-protein-coupled receptors in lung adenocarcinoma and colorectal carcinoma cell lines infected with SARS-CoV-2 and the effect of their knock-down on viral replication

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Adhesion G protein-coupled receptors (aGPCRs) play important role in neurodevelopment, immune defense, and cancer but their role during viral infections is mostly unexplored. We search for specific aGPCRs involved in the SARS-CoV-2 infection of mammalian cells in aim to characterize their interaction with viral proteins.
We infected human epithelial cell lines derived from lung adenocarcinoma (Calu-3) and cell line derived from colorectal carcinoma (Caco-2) with SARS-CoV-2, and analyzed changes in the mRNA level of the individual aGPCRs at 6 and 12 hours post-infection. Based on the significantly changed mRNA levels, four aGPCR candidates were chosen for further experiments – ADGRB3, ADGRD1, ADGRG7, and ADGRV1. ADGRD1 was identified as a common candidate as its mRNA levels were elevated upon infection in both Calu-3 and Caco-2 cell lines. Presumably, as a result of Calu-3 cell line´s better infectability by SARS-CoV-2, aGPCRs´ mRNA changes upon infection were in Calu-3 more prominent. Next, we performed a siRNA knock-down of selected four aGPCRs in both cell lines and analyzed its effect on SARS-CoV-2 entry, replication, and infectivity. We have observed significant decrease of newly released SARS-CoV-2 in culture media by RT-qPCR in both cells with down-regulated ADGRB3, ADGRD1, and ADGRV1. In addition, using plaque assay we observed slight reduction in SARS-CoV-2 infectivity in Calu-3 cells with down-regulated all four aGPCRs. In contrast, knock-down of ADGRB3, ADGRD1, ADGRG7, and ADGRV1 had no effect on SARS-CoV-2 entry.
In summary, our data suggest that selected aGPCRs might play a role during SARS-CoV-2 infection of mammalian cells.

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The adhesion GPCR ADGRL1/LPHN1 is involved in the regulation of food intake

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The increasing prevalence of obesity is one of the globally leading health issues. It contributes to major health problems such as type 2 diabetes or cardiovascular diseases, ultimately leading to increased morbidity and mortality. Thus, it is fundamental to understand the complex interplay of regulating food intake and energy balance for the successful implementation of pharmaceutical approaches to combat obesity. G protein-coupled receptors (GPCRs) are among the primary regulators of metabolism and energetic equilibrium, and have already been successfully targeted in therapies for various diseases. These receptors play a significant role in modulating various aspects such as appetite and satiety signals within specific hypothalamic neurons, as well as metabolizing glucose and lipids, along with the release of hormones from adipocytes.
A so far lesser-explored family of GPCRs, which have been shown to play a role in the regulation of metabolism, are the adhesion GPCRs (aGPCRs). For instance, within the aGPCRs, the Latrophilin subfamily is attributed a role in glucose-induced insulin secretion. In the present study, we have successfully pinpointed Latrophilin 1 (ADGRL1/LPHN1) as a significant component within the complex framework governing food intake and energetic balance. The absence of the receptor in mice leads to a notable rise in food consumption and consequently, pronounced obesity over time. Further, the animals suffer from a decreased glucose tolerance and insulin sensitivity as well as altered lipolysis. Remarkably, a human LPHN1 variant carrying a premature stop codon, thus truncating the aGPCR, was detected in obese patients, reinforcing the connection of the receptor with the regulation of body weight and energy balance. We show that this variant has different expression and signaling capacities. As a result, we speculate that the impaired functioning of LPHN1 represents a pivotal risk factor in the development of obesity.

ADGRG1/GPR56 Regulates Microglial Actin Dynamics and Phagocytosis to Limit Alzheimer's Disease Progression

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Alzheimer's disease (AD) is a devastating neurodegenerative disease with virtually no therapeutic interventions to reverse its pathology. Recent studies emphasize the role of glial cells, particularly microglia, in maintaining brain homeostasis and impacting AD progression. The adhesion G protein-coupled receptor GPR56 (also known as ADGRG1) is one of the critical genes defining "true" microglia: *Gpr56* is only expressed in yolk sac-derived microglia but not in fetal liver- and bone marrow-derived microglia-like cells, even after long-term adaptation in the central nervous system *in vivo* (Bennett et al., Neuron 2018). A recent study from Mathys *et al.* highlighted GPR56 as one of the top five genes upregulated in microglia in individuals with early-stage AD compared to those with no pathology or late-stage AD. Importantly, their data were generated from participants in a community-based cohort study, the Religious Order Study (ROS)/Rush Memory and Aging Project (MAP), collectively known as ROSMAP. Of those autopsied, the mean age was 89 years. This observation suggests that individuals with upregulated microglial GPR56 may have survived to an advanced age with mild AD pathology. In the present study, we test the hypothesis that microglial GPR56 prevents AD progression by employing both mouse models and human AD brain samples. Utilizing a new AD mouse model, in which microglial GPR56 is specifically deleted in 5xFAD mice (termed as AD-cKO and AD-control), we observed an exacerbated AD pathology in the absence of microglial GPR56. This was characterized by impaired microglial response, increased plaque burden, more severe neuronal pathology, and cognitive deficits. Single-nucleus RNA sequencing (snRNAseq) revealed a downregulation of genes linked to microglia homeostasis, phagocytosis, and lysosomal functions in AD-cKO microglia. Further in vitro and in vivo studies revealed microglial GPR56 regulate actin dynamics and phagocytosis of Aβ amyloids. In addition, our studies in human AD patients revealed the highest GPR56 expression in the microglia of individuals with mild cognitive impairments (MCI), compared to control and severe AD individuals. Pearson correlation coefficient analysis using several published human sequencing databases corroborated our results in mouse models. In conclusion, our study results support that microglial GPR56 plays an indispensable role in curtailing AD progression, presenting a potential new therapeutic target in combating AD.

Posters

Influence of the Cystic fibrosis phenotype on the expression and localization of latrophilin-2 in airway epithelial cells

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Latrophilin-2 (LPHN2, encoded by *ADGRL2*) is an adhesion GPCR known for its role in neuronal development and synapse formation. Emerging evidence indicate that it is widely distributed across tissues and versatile in various pathological contexts. The LPHN2 structure typically comprises a C-terminal signaling domain and an extended N-terminal extracellular domain, the latter mediating complex interactions with cell surface molecules and extracellular matrix components. Among the four families of adhesion motif-containing transmembrane proteins, fibronectin leucine-rich transmembrane proteins were described as high-affinity endogenous ligands for LPHNs. The unique structural properties of LPHN2 let us investigate its role in cystic fibrosis (CF), an autosomal recessive severe respiratory disease characterized by loss of apicobasal polarity of the airway epithelium and ectopic surface deposition of fibronectin. Analysis of RNA-seq performed on primary cultures of airway epithelial cell from healthy subjects and CF patients predicted enrichment of *ADGRL2* in CF. We investigated the expression and distribution of LPHN2 using imaging techniques in primary cultures and in a lab-created airway epithelial cell line model of CF. Although the study on primary cells is still in progress, preliminary data indicate lower LPHN2 expression in the CF cell line, with a higher abundance of the protein on the plasma membrane compared to control cells. No differences in the mRNA levels were observed. This suggests that, in the CF condition, LPHN2 might be recruited to the cell membrane because of interaction of its N-terminal domain with the modified microenvironment. Screening for LPHN2 ligands could prove as an effective strategy for understanding the localization of LPHN2 and exploring its implicated signaling pathways in CF. This study introduces a novel perspective on the possible involvement of LPHN2 in CF pathogenesis and may shed light on its potential as an unexplored therapeutic target for addressing the multifaceted challenges posed by the disease.

GPR126/ADGRG6 Arrestin Interaction (lecture and poster)

Lisa Kupsch, Lusia Riedel, Ines Liebscher, Sandra Berndt

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2 Wilde C, Chaudhry PM, Luo R, Simon KU, Piao X, Liebscher I. Collagen VI Is a Gi-Biased Ligand of the Adhesion GPCR GPR126/ADGRG6. Cells. 2023 Jun 5;12(11):1551. (doi: 10.3390/cells12111551)
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Adhesion GPCR, GRP126/ADGRG6 is activated by different extracellular matrix proteins, prion proteins or mechanical forces and mediates Gs or Gi signaling pathways [1,2]. Both pathways have different regulatory roles, like the differentiation of adipocytes, osteoblasts, and Schwann cells and on the other hand breast cancer proliferation. Next to classical G protein-mediated signaling cascades, GPCR stimulation can result in the activation of various mitogen activated protein kinases (MAPK). The impact of non-canonical signaling in these functions remains elusive. The activation of the downstream effectors like Src kinases and ERK1/2 has been reported for GPR126/ADGRG6 [3,4]. Nevertheless, the activation mechanism of the effectors as well as the functional role is not well understood. Often the activation occurs via scaffold proteins like arrestins. Here, we investigate GPR126/ADGRG6 arrestin interaction and its role in signaling bias. First, we showed the overall binding of arrestin to the receptor using Co- immunoprecipitation. We could detect a more robust binding of the CTF GPR126, which represents an activated form of the receptor. Next, we detected the receptor arrestin interaction using the NanoBiT complementation assay. Arrestins are known to bind to phosphorylated serine/threonine cluster. For the identification which phosphorylation pattern is present and crucial for arrestin binding, we are introducing systematic point mutations. GPR126/ADGRG6 has three potential phosphorylation cluster which we will analyze. In the field, it is discussed if the phosphorylation position is impacting the arrestin activation differently, which could cause a selectivity for specific effectors and therefore signaling cascades.

The adhesion G protein-coupled receptor GPR56/ ADGRG1 in cytotoxic lymphocytes

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GPR56/ADGRG1 is an adhesion G protein-coupled receptor connected to brain development, hematopoiesis, male fertility, and tumorigenesis. Nevertheless, expression of GPR56 is not restricted to developmental processes. Studies over the last years have demonstrated a marked presence of GPR56 in human cytotoxic NK and T cells. Expression of GPR56 in these cells is driven by the transcription factor HOBIT, corresponds with the production of cytolytic mediators and the presence of CX3CR1 and CD57, indicates a state of terminal differentiation and cellular exhaustion, and disappears upon cellular activation. Functional studies indicate that GPR56 regulates cell migration and effector functions and thereby acts as an inhibitory immune checkpoint. We here discuss the current state of knowledge regarding GPR56 in cytotoxic lymphocytes.

Oxime blot: a novel method for reliable and sensitive detection of carbonylated proteins in diverse biological systems

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Oxidative stress and the ensuing oxidative protein damage occur in various biological processes and diseases. The carbonyl group on amino acid side chains is the most widely used protein oxidation biomarker. Carbonyl groups are commonly detected indirectly, through their reaction with 2,4-dinitrophenylhydrazine (DNPH) under low pH conditions and further labeling with an anti-DNP antibody. However, the DNPH immunoblotting method lacks protocol standardization, exhibits technical bias, and has low reliability. To overcome these shortcomings, we have developed a new blotting method in which the carbonyl group reacts with the biotin-aminooxy probe to form a chemically stable oxime bond. The reaction speed and the extent of the carbonyl group derivatization are increased by a p-phenylenediamine (pPDA) catalyst under neutral pH conditions. This protocol improvement is crucial because it ensures that the carbonyl derivatization step reaches a reaction plateau and increases the sensitivity and robustness of protein carbonyl detection. Furthermore, derivatization under pH-neutral conditions ensures a good SDS-PAGE protein migration pattern and minimizes protein loss while being compatible with protein immunoprecipitation. This work describes the new Oxime blot method and demonstrates its use in the detection of protein carbonylation in complex matrices from diverse biological samples.

Ladouce R*, Combes, G.F.*, et al. Oxime blot: A novel method for reliable and sensitive detection of carbonylated proteins in diverse biological systems. Redox Biology (2023). https://doi.org/10.1016/j.redox.2023.102743

Molecular Dynamics of Adhesion GPCR shape neurophysiology

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All Adhesion GPCR share the evolutionarily conserved GPCR autoproteolysis inducing (GAIN) domain, which harbors a tethered agonist, the *Stachel*, potent to trigger receptor activation. Interestingly, recent Molecular Dynamics simulations predicted that the GAIN domain contains two flexible regions, termed flap 1 and flap 2. Those flaps shield the *Stachel* and are able to expose it through lateral movement, therefore increasing solvent accessibility and potentially enabling receptor activation. However, the physiological effects of such flap movements remains unknown.

To further investigate this mechanism *in vivo*, we mutated specific GAIN domain residues of Cirl, the Latrophilin-1 homologue in *Drosophila melanogaster*. The GAIN domain residues were selected based on Molecular Dynamics simulations with the goal to alter flap flexibility, *i.e.* Stachel exposure. Cirl is expressed in sensory neurons where it helps to shape mechanosensation by suppressing cAMP levels and modulating ion fluxes through transient receptor potential (TRP) channels.

To investigate effects of GAIN domain mutations on neuron function, we will combine behavioral and functional readouts. First experiments revealed differences in Cirl expression and autoproteolysis, which may be linked with the locomotion deficits observed in some GAIN-flap mutants. Our ultimate goal is to further understand the mechanics of flap mobility and its consequences for expression and functionality of Cirl *in vivo* but to also expand our general knowledge on activation mechanisms of aGPCR.

CELSR2 is a potent biomarker and druggable target in triple negative breast cancer

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Abstract : Breast cancer is the most prevalent malignancy in modern times around the world. Molecular studies identified different subtypes based on the expression of hormone receptors, of which are estrogen (ER) and progesterone (PR) receptors and human epidermal receptor 2 (HER2). The subtype negative for all three receptors known as the triple negative breast cancer is the most aggressive, highly metastatic and is the deadliest as of today, with 10% of metastatic patients survive over 5 years. The lack targeted therapies limits the clinicians' options, thus we need to identify potent and druggable biomarkers. Here we identify CELSR2, an adhesion GPCR overexpressed in breast tumors and is associated with a higher occurrence of metastasis. Within breast tumors, at the single cell level, CELSR2 is predominantly expressed on tumor cells. Functionally, transient knockdown of CELSR2 impairs cell capability to adhere to extracellular matrix, migrate and invade the extracellular matrix in 3D settings. Moreover, we link this impairment to the signaling of CELSR2 through the PKA-Creb axis to promote cell metabolism. Overall, the present study provides a novel insight on the role and mechanism of adhesion GPCRs and provides evidence of the potential effects upon drugging.

Combination of Oxidoreductases and Electrodes for Application in Enzymatic Biosensors and Biofuel Cells

Martina Perić Bakulić, Emmanuelle Bignon, **Željka Sanader Maršić***

In nature, enzymes from the oxidoreductase class transfer electrons from one molecule to another in a cellular environment where they are free to move. The source of electrons are small biomolecules (e.g. sugars). With the goal of replacing metal electrodes with more environmentally friendly products, these enzymes offer a solution and are a key ingredient in enzymatic biosensors and fuel cells (EFCs). The current challenge is to find the optimal combination of enzyme and material for the electrode that provides the greatest transfer of electrons.

In this poster, we will summarize the characteristics of immobilized enzymes on the surface of electrodes made of different materials (e.g. carbon materials). Goal is to understand the electron transfer process in detail, step by step, at the atomic level. This would establish a methodology for the rational design of enzymatic biosensors and biofuel cells.