COST Action
CA18133
ERNEST
European Research
Network on Signal
Transduction

Horizon 2020
Programme

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EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

BOĞAZİÇİ ÜNİVERSİTESİ
1863

2nd ERNEST Online Meeting

New Perspectives in Signal Transduction:
GPCRs and Beyond

28-30 March, 2020

Abstract Book

Greetings!

It is our pleasure to host you **online** for Second Meeting of the European Research Network on Signal Transduction (**ERNEST** COST Action CA18133) with the theme of ***New Perspectives in Signal Transduction: GPCRs and Beyond*** March 28-30, 2020.

The main scientific objective of **ERNEST** is to develop a common, comprehensive and holistic map of signal transduction that will advance development of pathway-specific chemical modulators. This unique and innovative goal will be realized by linking diverse research groups in the field through the networking activities.

In line with the scientific objectives of the ERNEST, the program of the meeting will cover different aspects of signal transduction involving, but not limited to, GPCRs: Macromolecular interactions in signaling pathways, biological roles of signal transduction, molecular modulators of signal transduction, advances in methodologies and technologies and public web resources.

This 3-day event will bring together internationally renowned scientists as well as early career researchers from all around Europe and nearby countries. It will serve as an excellent global platform for researchers from industry and academia to interact and discuss the latest scientific findings, innovations and technologies in the field of cellular signalling. We believe that the meeting will also be an exceptional stage for *virtual* congregation, *streaming* of ideas and establishing new *Skype-enabled* collaborations.

We are excited to be part of this event, and we hope that you will be able to participate and join us in this effort.

<u>Organizing Committee</u>

İbrahim Yaman (Local Organizer, Boğaziçi University)
Necla Birgül (Local Organizer, Boğaziçi University)
Martha Sommer (Chair of ERNEST, Charité and MDC Berlin)
Jana Selent (Vice-chair of ERNEST, Pompeu Fabra University)

2nd ERNEST Meeting: Online program		
Time (CET)	**Day 1: Saturday, 28 March 2020**	Abstract page #
9:00-9:45	**Opening and Announcements** **Ibrahim Yaman, Necla Birgul, Martha Sommer**	
10:00-10:45	**Keynote talk** **Michel Bouvier** (University of Montreal) *Detecting and analyzing functional selectivity and biased signalling, historical perspective and potential for drug discovery*	6
10:45-11:15	**BREAK (30 min)**	
11:15-12:05	**SESSION 1 WG1** (chair: **Milka Vrecl**) 11:15-11:35 **Martha Sommer** (Charité Medical University Berlin) *Dynamic versatility of arrestin in GPCR and membrane binding* **Short talks** (selected from abstracts) 11:35 - 11:50 **Daniel Hilger** (Philipps-University Marburg) *Structural insights into differences in G protein activation by Family A and Family B GPCRs* 11:50 - 12:05 **Gemma Navarro** (University of Barcelona) *Functional pre-coupled complexes of receptor heteromers and adenylyl-cyclase*	7 8 9
12:05-12:30	**BREAK (25 min)**	
12:30-13:00	**SESSION 2** **Poster Flash Talks 1 to 10** (10 x 3 min)	10-19
13:00-14:30	**BREAK (90 min)**	
14:30-15:30	**SESSION 3 Short Talks** (chair: **Fabrizio Fierro**) 14:30 - 14:45 **Johanna K. S. Tiemann** (University of Copenhagen) *Formation of a β2AR*-GsGDP intermediate complex* 14:45 - 15:00 **Thor Moller** (University of Copenhagen) *Dissecting the roles of GRK2 and GRK3 in μ-opioid receptor internalization and b-arrestin2 recruitment using CRISPR/Cas9-edited HEK293 cells* 15:00 - 15:15 **Aleksandra Luginina** (Moscow Inst. Physics&Technology) *δ-branch of class A GPCRs: structural aspects of activation* 15:15 - 15:30 **Pedard Martin** (Normandie University) *The urotensin II G protein-coupled receptor relays key neurobiological mechanisms in subarachnoid hemorrhage through a Gq-dependent pathway*	20 21 22 23
15:30-15:45	**BREAK (15 min)**	
15:45-17:15	**SESSION 4 GPCRdb / GPCRmd satellite meeting** (organizers: Jana Selent and David Gloriam) 15:45 - 16:30 GPCRdb (**Albert Kooistra & Christian Munk**) · Demo-session (www.gpcrdb.org) · Questions and user-implementation wishes 16:30 - 17:15 GPCRmd (**Jana Selent**) · Demo-session (www.gpcrmd.org) · Updates on objectives and call for contributions	24
17:15-18:00	**BREAK (45 min)**	
18:00-20:00	**SESSION 5** **Social event - meeting up in virtual rooms**	25

2nd ERNEST Meeting: Online program		
Time (CET)	**Day 2: Sunday, 29 March 2020**	Abstract page #
9:00-10:30	**SESSION 6 WG2** (chair: **Necla Birgul & Nina Vardjan**) 9:00-09:20 **Miriam Stoeber** (University of Geneva) *Biosensors reveal ligand-selective patterns of opioid receptor activation* 9:20-9:40 **Graham Ladds** (University of Cambridge) *Systems analysis of G protein-coupled receptor pharmacology* 9:40-10:00 **Ines Liebscher** (University of Leipzig) *The force within – Mechano-sensitive adhesion GPCR shape physiology* **Short talks** (selected from abstracts) 10:00-10:15 **Hélène Castel** (Normandie University) *Chemokine GPCRs function with different G proteins or anchoring partners to control glioma invasiveness* 10:15-10:30 **Aida Shahrakia** (Bogazici University) *In silico and in vitro Approaches to Characterize a Novel G-Protein Coupled Receptor in Thaumetopoea pityocampa*	26 27 28 29 30
10:30-11:20	**BREAK (50 min)**	
11:20-12:45	**SESSION 7 WG3** (chair: **Masha Niv & Fabrizio Fierro**) 11:20-11:40 **Christa Müller** (University of Bonn) *Tools and drugs for G proteins and G protein-coupled receptors* 11:40-12:00 **Leigh Stoddart** (University of Nottingham) *Ligand-directed affinity labelling - a new approach to visualise GPCRs* **Short talks** (selected from abstracts) 12:00 - 12:15 **Giulia Morra** (Ist di Scienze e Tecnologie Chimiche) *Investigating functional selectivity in and around GPCRs with Molecular Dynamics* 12:15 - 12:30 **Pierre Matricon** (Uppsala University) *Design of a GPCR agonist by Targeting a Binding Site Water Network* 12:30 - 12:45 **Vigneshwaran Namasivayam** (University of Bonn) *Computational approaches for the proinflammatory lipid-activated G protein-coupled receptor GPR84 providing structural insights*	31 33 34 35 36
12:45-13:30	**BREAK (45 min)**	
13:30-14:00	**SESSION 9** **Poster Flash Talks 11 to 19** (9 x 3 min)	37-45
POSTPONED	**SESSION 8** Academia-Industry Cooperation	
POSTPONED	**SESSION 10** Management Committee Meeting	

2nd ERNEST Meeting: Online program		
Time (CET)	**Day 3: Monday, 30 March 2020**	Abstract page #
9:00-10:40	**SESSION 12 WG4** (chair: **Nuska Tschammer & Eddy Sotelo**) 9:00-9:50 **Martin J. Lohse** (MDC, Berlin; ISAR Bioscience Institute, Munich) *Watching receptors at work – using the microscope to study GPCRs* 9:50-10:10 **Bence Szalai** (University of Semmelweis) *Gene expression signatures - footprints of GPCR signalling pathway activity* **Short talks** (selected from abstracts) 10:10-10:25 **Adrian Morales-Pastor** (Pompeu Fabra University) *Exploration of allosteric communication networks that underlie GPCR functionality* 10:25-10:40 **Tõnis Laasfeld** (University of Tartu) *Novel fluorescence ligands for the characterization of muscarinic acetylcholine receptors*	46 47 48 49
10:40-11:15	**BREAK (35 min)**	
11:20-12:30	**SESSION 13 WG5** (chair: **Jana Selent**) 11:15-11:45 **Vassiliki Iconomidou** & **Avgi-Elena Apostolakou** (University of Athens) *hGPCRnet: A tool for Cell-Type Specifc Analysis of GPCR Signaling Pathways* 11:45-12:10 GPCRdb update (**David Gloriam**) 12:10-12:30 GPCRmd update (**Mariona Torrens**)	50 51 52
12:30-12:45	**BREAK (15 min)**	
12:45-13:45	**SESSION 14 Short talks** (chair: **David Gloriam**) 12:45-13:00 **Alexey Bondar** (Augusta University) *Membrane domain localization and mobility of Gs signaling cascade components* 13:00-13:15 **Marcel Bermudez** (Freie Universität Berlin) *Pharmacological approaches to decipher the signaling networks of GPCR of the reproductive axis* 13:15-13:30 **Pascale Crépieux** (Université de Tours) *Pharmacological approaches to decipher the signaling networks of GPCR of the* reproductive axis 13:30-13:45 **Shannon O'Brien** (University of Birmingham) *Hydroxy-carboxylic acid receptor 2 signalling at endosomal compartments*	53 54 55 56
13:45-14:00	**BREAK (15 min)**	
14:00	**SESSION 15** **Meeting Closure** (chair: Martha Sommer)	57
POSTPONED	**Focus Group on Functional Selectivity** (by invitation only) Organizer: Peter Kolb	

KEYNOTE LECTURE

Saturday, March 28, 10:00

Detecting and analyzing functional selectivity and biased signalling, historical perspective and potential for drug discovery

Michel Bouvier

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Principal Investigator
Email address of the presenter: michel.bouvier@umontreal.ca

It is now clear that G protein-coupled receptors (GPCRs) are not unidimensional switches that turn 'on' or 'off' a single signaling pathway. Instead, each receptor can engage multiple signaling partners that can engage various downstream effector systems. Individual ligands can have differential efficacies toward specific subsets of the signaling effector repertoire engaged by a given receptor. This phenomenon, known as ligand-biased signaling or functional selectivity, opens new opportunities for the development of drugs targeting therapeutically relevant pathways while sparing those leading to undesirable effects. Yet, this added level of complexity raises important questions on how to best establish functional selectivity/biased signaling and to quantify it. What type of assays should be used or avoided? What are the best models to quantify the extent of bias? how to select the reference compounds? How to translate functional selectivity observed in cultured cell systems into physiological context to assess its true therapeutic potential? In an attempt to stimulate discussions bearing on these questions, the talk will present an historical perspective on the discovery of bias. I will also present some of the assays that can facilitate detection of biase and discuss the difficulties and different solutions related to the quantification of bias in a meaningful manner. Finally, the physiological relevance and therapeutic potential of functional selectivity and biased signaling will be discussed in the context of published and unpublished examples.

Dynamic versatility of arrestin in GPCR and membrane binding

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Principal Investigator/Group Leader
Email address of the presenter: martha.sommer@charite.de

G-protein-coupled receptors are membrane proteins that are regulated by a small family of arrestin proteins. During formation of the arrestin–receptor complex, arrestin first interacts with the phosphorylated receptor C terminus in a pre-complex, which activates arrestin for tight receptor binding. Currently, little is known about the structure of the pre-complex and its transition to a high-affinity complex. We carried out molecular dynamics simulations and site-directed fluorescence experiments on arrestin-1 interactions with rhodopsin, showing that loops within the C-edge of arrestin function as a membrane anchor. Activation of arrestin by receptor-attached phosphates is necessary for C-edge engagement of the membrane, and we show that these interactions are distinct in the pre-complex and high-affinity complex in regard to their conformation and orientation. These findings will be discussed in light of the surprisingly distinct arrestin-GPCR complex structures that have been recently published.

Structural insights into differences in G protein activation by Family A and Family B GPCRs

Daniel Hilger, Kaavya Krishna Kumar, Hongli Hu, Mie Fabricius Pedersen, Evan S. O'Brien, Lise Giehm, Christine Jennings, Gözde Eskici, Michael Lerch, Jesper Mosolff Mathiesen, Georgios Skiniotis, Brian K. Kobilka

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Section:

6. Macromolecular Interactions in Signalling Pathways,
7. Biological Roles of Signal Transduction,
8. Molecular Modulators of Signal Transduction,
9. Advanced Methodologies and Technologies
10. Public Web Resources

Position of the presenter: Group leader
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Family B G protein coupled receptors (GPCRs) play important roles in carbohydrate and mineral metabolism. Recent structures of Family B GPCR-G_s protein complexes reveal a disruption in the α-helix of transmembrane segment 6 (TM6) not observed in Family A GPCRs. To investigate the functional impact of this structural difference, we compare the structure and function of the glucagon receptor (GCGR, Family B) and the β_2 adrenergic receptor (β_2AR, Family A). We determined the structure of the GCGR- G_s complex, which show the distinct break in TM6. Functional studies revealed much slower rates for G protein activation by the GCGR compared to the β2AR. Biophysical studies provide evidence this difference can be attributed to the energetically unfavorable structural change in TM6 characteristic of Family B GPCRs.

Functional pre-coupled complexes of receptor heteromers and adenylyl-cyclase

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Group Leader
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G protein-coupled receptors (GPCRs), G proteins and adenylyl cyclase (AC) compose one of the most studied transmembrane cell signaling pathways. Still controversial is the ligand-dependent mode of interactions between these signaling molecules: random collision of freely mobile membrane molecules or rearrangement of pre-coupled elements in a macromolecular complex. Also controversial is the fact that a GPCR homodimer coupled to a single heterotrimeric G protein constitutes a common functional unit. Using synthetic peptides that target putative oligomerization transmembrane interfaces of GPCR homo and heterodimers and GPCRs and AC, we provide strong evidence for the existence of functional pre-coupled complexes of two different homodimers coupled to their cognate Gs and Gi proteins and to AC. We also demonstrate that this macromolecular complex provides the necessary frame for the canonical Gs-Gi interactions at the AC level, the ability of a Gi-coupled GPCR to counteract AC activation induced by a Gs-coupled GPCR.

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Combined ligand-based and structure-based approaches in rational drug design of novel 5-HT$_{2A}$ receptor antagonists #1

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Group Leader
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The serotonin 5-HT$_{2A}$ receptor plays an important role in many physiological functions.[1] Various neurological and psychiatric disorders are associated with certain imbalances in 5-HT neurotransmission.[2] Therefore, antagonists of these receptors are efficient as antipsychotics but also have a significant role in the treatment of depression, anxiety, and Parkinson disease. In order to find crucial structural features responsible for high binding affinity of 5-HT$_{2A}$ antagonists, we have combined structure based and ligand based drug design methods. This study was performed on wide range of structurally diverse antagonists. Based on their chemical structures they were divided into three different clusters: clozapine, ziprasidone, and CHEMBL240876 derivatives. Each cluster representative in complex with 5-HT$_{2A}$ receptor was submitted to 50ns long molecular dynamics (MD) simulation in order to obtain their inactive, antagonist-bound, conformations. Subsequently, these conformations were used as templates for docking studies in order to generate virtually bioactive conformations of studied ligands. Selected conformers were used for calculation of specific molecular descriptors (Grid Independent Descriptors- GRIND) and three-dimensional quantitative structure-activity relationship (3D-QSAR) model building. The 3D-QSAR approach helps us to identify the most important structural determinants responsible for the antagonistic activity and to propose structural modification for novel antagonists of serotonin 5-HT$_{2A}$ receptors. Furthermore, the reliability and predictive potential of the created model was evaluated using an external test set compounds. Results obtained from performed 3D-QSAR, MD and molecular docking studies were analysed and used for rational drug design of novel 5-HT$_{2A}$ receptor antagonists.

References

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The New Paradigm of Intracellular GPCR Signalling in Metabolic Diseases #2

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: PhD student
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The free fatty acid 4 receptor (FFAR4) is highly expressed in tissues involved in metabolic homeostasis, including adipose, where its pharmacological stimulation improves glucose uptake, insulin sensitivity and inhibits lipolysis (1). Work by our group, and others, have revealed that GPCRs are not only active at the plasma membrane, as originally thought, but can also be active at intracellular sites - for example the early endosomes or the Golgi complex/trans-Golgi network (2,3). The physiological relevance of this intracellular signalling phenomenon is at present largely unknown, especially when considering metabolically relevant GPCRs e.g. the FFA4R.

Here, we used highly-inclined and laminated optical sheet (HILO) (4) microscopy and bioluminescence resonance energy transfer (BRET) (5) to elucidate the relationship between FFAR4 (short isoform) internalisation, trafficking and signalling. Our results indicate that the FFA4R rapidly internalises to Rab5 positive endosomes and other intracellular compartments upon stimulation with the full agonist TUG-891. Subsequently, the FFA4R recruits subtype specific mini Gα protein probes (6) to intracellular membranes, indicating that the FFA4R remains active at intracellular sites after stimulation. Further experiments will investigate the consequences of intracellular FFA4R signalling downstream of G protein activation and its implications in adipocyte metabolism. Understanding the mechanisms and relevance of intracellular signalling by the FFAR4 and other metabolically relevant GPCRs could ultimately pave the way to novel therapeutic strategies for metabolic diseases.

References

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Structural Insights into the Pharmacological Action of a Functionally Selective GLP-1R agonist #3

Wijnand J.C. van der Velden[1], Florent X. Smit[1], Charlotte B. Christiansen[2,3], Thor C. Møller[4], Olav Larsen[1], Sine P. Schiellerup[1], Hans Bräuner-Osborne[4], Jens J. Holst[2,3], Bolette Hartmann[2,3], Thomas M. Frimurer[5], Mette M. Rosenkilde[1]

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: PhD student
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Glucagon-like peptide-1 receptor (GLP-1R) ligands that selectively confer activity in one pathway over another are useful tools to understand the biological consequences of functional selectivity and thereby reveal how to reduce on-target site effects. In the present study, we describe how complementary alterations in GLP-1 and its receptor (GLP-1R) alter the functional output. Despite similar affinities (pK_D values of 8.3 and 8.2) and efficacies in G protein signaling (cAMP production), an analog of GLP-1(7-36)NH_2 dramatically impaired the recruitment of β-arrestin and receptor internalization. Moreover, the GLP-1 analog induced a significantly lower insulin and somatostatin secretion determined, but similar glucagon-lowering effect in a perfused pancreas model in rat. This altered function was driven by a lower B_{max} (up to 15.3-fold) and altered ligand-receptor binding kinetics with a slower observed association (k_{obs}; 1.9-fold) and faster initial dissociation rate ($k_{off\ fast}$; 2.5-fold) resulting in a lower residence time (RT) for the analog. Molecular dynamics simulation and receptor mutagenesis revealed fewer hydrogen bond interactions of the GLP-1 analog with the GLP-1R compared to GLP-1. By comparing the impact on GLP-1-mediated signaling of 27 receptor mutations; two hot spots (1: F187$^{2.57}$, R190$^{2.60}$, N240$^{3.43}$ and E364$^{6.53}$; 2: N182$^{2.52}$, W243$^{3.46}$ and W274$^{4.50}$) were identified in the GLP-1R with selective importance for arrestin recruitment over cAMP production. As the GLP-1 analog's functional selectivity pointed toward altered ligand-receptor binding kinetics, based on different ligand binding and receptor conformations, these parameters are highlighted to be essential for the incretin effect of GLP-1.

GPR101 orphan receptor: a novel cause of growth hormone deregulation #4

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Section:

1. Macromolecular Interactions in Signaling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Postdoc
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GPR101 is an orphan (without known ligand) G-protein coupled receptor. Recently, a clinical study showed that GPR101 is strongly associated X-linked acrogigantism syndrome (XLAG), which is a genetic rare disorder caused by Xq26.3 microduplications and characterized by an abnormal growth hormone (GH) hypersecretion **(1)**. Considering the GPR101 involvement in X-LAG on one hand, and the lack of pharmacological tools to investigate its function and/or to correct its defects on the other hand, we propose a research program to study GPR101 signaling pathways and its role in GH regulation.
GPR101 is characterized by a very high level of constitutive activity. Therefore, we analyzed GPR101 constitutively activated signaling pathways. We confirmed an increase of cAMP levels and observed a strong association with arrestin pathway. We completed our study with an examination of receptor coupling to other pathways and G proteins. With confocal microscopy and FACS analysis, we determined the receptor precise cellular localization and constitutive trafficking. Furthermore, we applied targeted mutagenesis to modulate the receptor constitutive activity in order to understand the receptor function at a molecular level. These GPR101 mutants will help us to understand the role of this receptor in GH regulation and/or to treat people suffering from pituitary dysfunction. These information are an absolute prerequisite to link molecular pharmacology of GPR101 with physiological functions.

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The Effects of CDP-Choline on Autophagy and Mitochondrial Dynamics in Amyloid-Treated PC12 Cells #5

Beki Kan[1], Begum Bilge[1], Suleyman Bozkurt[1], Ismail Hakki Ulus[2] and Devrim Oz-Arslan[1]

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Group leader
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Recent studies suggest that autophagy may have a crucial role in Alzheimer's disease (AD). Cytidine-5'-diphosphocholine (CDP-Ch), an intermediate in the biosynthesis of membrane phospholipids, is known to have neuroprotective effects in several diseases but the mechanism remains unclear. In this study, we aimed to understand the effect of CDP-Ch treatment on autophagy and mitochondrial dynamics during amyloid-beta ($A\beta_{1-42}$) mediated neuronal injury. To this end, nerve growth factor (NGF)-differentiated PC12 cells were treated with $A\beta_{1-42}$ in the presence and absence of CDP-Ch. We examined the levels of several autophagic markers, including LC3B, p62, and Beclin 1 and also Mitofusin-2 (Mfn2), an outer mitochondrial membrane GTPase involved in mitochondrial fusion by immunoblotting. Mitochondrial membrane potential (MMP) and mitochondrial mass were evaluated by flow cytometry and confocal imaging after probing with mitochondria-specific dyes. Oxygen consumption rate (OCR) was measured using Agilent Seahorse XFP Cell Mito Stress Kit.
We observed increases in LCB3 and Mfn-2 levels of differentiated PC12 cells upon CDP-Ch treatment. Although CDP-Ch treatment did not cause a change in MMP, mitochondrial respiration was reduced. $A\beta_{1-42}$ treatment resulted in increased levels of LC3B and Beclin 1. CDP-Ch pre-treatment of injured cells reduced MitoSox levels.
Our preliminary results suggest that CDP-Ch treatment and amyloid beta injury affect autophagy and mitochondrial function in NGF- differentiated PC12 cells. An understanding of the role of CDP-Ch in autophagy and mitochondrial dynamics may shed light into its neuroprotective effects.

This work is supported by The Scientific and Technological Research Council of Turkey (Grant number:114Z494).

Differential G-protein regulation elicited by seven serotonin 2A receptor (5-HT2AR) "antagonist" drugs in *postmortem* human brain #6

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Research Scientist
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Based on cell culture assays, it has been proposed that different 5-HT2AR antagonists really act as 5-HT2AR inverse agonists. Additionally, information on the different signalling pathways involved in this antagonism/inverse agonism is scarce.
The aim of the present work is the characterization of the functional profile of selective 5-HT2AR inverse agonist/antagonist drugs (altanserin, eplivanserin, ketanserin, MDL11,939, nelotanserin, pimavanserin and volinanserin) in the coupling of 5-HT2ARs to Gαq/11- and Gαi1-proteins in *postmortem* human brain cortex membrane homogenates.
Concentration-response of [^{35}S]GTPγS binding assays coupled to immunoprecipitation with antibodies were carried out for the different 5-HT2AR compounds. In order to confirm the role of 5-HT2AR in the observed effects, one-point concentration (10 μM) experiments in the presence of MDL11,939 were performed in human cortex, and both WT and 5-HT2AR KO mice brain tissue.
MDL11,939 was the only tested drug showing no effect on basal activity of 5-HT2AR coupling to Gαi1- and Gαq/11-proteins. Both altanserin and pimavanserin displayed preferential inverse agonist effect on 5-HT2AR coupling to Gαi1-proteins, with no effect on Gαq/11-proteins, while volinanserin showed 5-HT2AR-mediated inverse agonist effect on Gαi1-, but also on Gαq/11-proteins. Eplivanserin and nelotanserin displayed inverse agonist properties on Gαq/11- and/or Gαi1-proteins that were not sensitive to MDL11,939, and were present in 5-HT2AR KO mice. Surprisingly, ketanserin behaved as a 5-HT2AR partial agonist on the Gαq/11-mediated signalling pathway.
These findings demonstrate the presence of constitutively active 5-HT2ARs in native brain tissue and show the differential targeting of G-protein signalling modulation by different 5-HT2AR drugs in *postmortem* human prefrontal cortex.

Novel Alzheimer's disease treatment: repurposing antipsychotics to compounds for neuron protection, integrity and growth #7

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Group Leader
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We used rational structure-based design methods to develop multi-target ligands of aminergic GPCRs which can be used to treat mental diseases. Detailed investigation of *in vitro* and *in vivo* profiles of the selected compounds indicated that they may be repurposed for the treatment of neurodegenerative diseases such as Alzheimer's disease.

It was determined that the selected compounds increase the proliferation of mouse hippocampal neuronal cells (cell line HT-22) and do not increase proliferation of neuroblastoma cells (cell line SH-SY5Y) based on the MTT assay. The neuronal cells incubated with the compounds are elongated with longer dendrites. Moreover, the compounds diminish excitotoxicity by decreasing concentration of Ca^{2+} ions in the cells and protect the neuronal cells against high temperature. Next, the compounds have antioxidant properties as they decrease the concentration of both reactive oxygen species and reactive nitrogen species in the cells. Importantly, the compounds exhibit pro-cognitive properties in passive avoidance test, modified elevated plus maze test and novel object recognition test in mice models, both after acute and chronic administration. The compounds have no significant toxic effect except for some hepatotoxicity. However, no damage at the subcellular level was caused by the studied compounds.

We performed *in silico* studies to decipher the possible molecular mechanisms underlying the observed activities of the investigated substances. PASS software indicated that neuron growth and dendrite elongation can be caused by activation of CAMK1 kinase. This hypothesis was confirmed using Western blot approach: we found that the studied compounds cause increase in CaMK1 protein expression.

Modulation of Structural and Dynamical Properties of GPCR Oligomers via Heterobivalent Ligands: The Case Study of Adenosine 2A Receptor /Dopamine 2 Receptor Tetramer #8

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of a presenter: Group Leader
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Oligomerization phenomenon is curical in the GPCR family as the functional state of a receptor in tis monomeric form might be modulated in the presence of other members in the oligomer. For instance, Adenosine 2A receptor ($A_{2A}R$) imparts antagonistic effect on Dopamine 2 receptor (D_2R) upon formation $A_{2A}R/D_2R$ dimer [1], which is a well-known allosteric interaction that causes alleviation of action of L-DOPA that is used to increase D_2R signaling in Parkinson's Disease (PD). In this respect, $A_{2A}R$ antagonists have emerged as promising candidates to prevent this allosteric interaction between $A_{2A}R$ and D_2R. Recently, $A_{2A}R$ and D_2R have been shown to form a tetramer in the *striatum* and simultaneous occupation of the $A_{2A}R$ pair within that tetramer by $A_{2A}R$ antagonist/agonist has been shown to increase D_2R signaling more compared to administration of $A_{2A}R$ antagonist alone [1]. Therefore, we are motivated by these findings to develop a library of heterobivalent ligands (HL) *in silico*, which are composed of $A_{2A}R$ agonist/antagonist. To this end, we first modeled the tetramer and docked HLs. We performed accelerated molecular dynamics simulations using explicit water and membrane representation. Our results showed that $A_{2A}R$ could be more effectively blocked in the presence of HL compared to systems where $A_{2A}R$ antagonist and agonist were not attached with a linker. To the best of our knowledge, this is the first study where the impact of a HL on dynamics of GPCR oligomer is studied, hence it provides a framework for development of effective HLs in future drug discovery studies.

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Identification of Allosteric Modifiers of Mosquito Odorant Receptor Function and Odor- Triggered Behaviors #9

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Senior Research Scientist
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Insect odorant receptors (ORs) are heteromeric ion channels each consisting of a common subunit, ORco, and one of multiple variable subunits, ORx, to which odorants acting as specific receptor agonists or antagonists bind. In cells expressing the ORco subunit alone, ORco forms functional homomeric channels that are activated by specific ORco agonists. We have previously shown that mosquito repellents that block odor-specific responses of multiple Anopheles gambiae ORs, act as ORco-specific antagonists, thus allosteric antagonists for the heteromeric receptors. Additionally, the responses of heteromeric receptors induced by ORx- agonists are enhanced in the presence of an ORco-agonist (OA), a finding suggesting that OA binding causes structural rearrangements in liganded ORx heteromers.
We are now reporting on the use of an insect cell-based ORco expression platform that allows the fast screening of compound collections for discovery of A. gambiae ORco agonists and antagonists. Screening of a small collection of volatile organic compounds (VOCs) of natural origin for relevant bioactive ligands resulted in the identification of several hits acting as ORco antagonists. We have established the functional relevance of the identified ORco hits using repellence assays against the tiger mosquito Aedes albopictus, which is capable of vectoring a number of infectious agents. Newly identified ORco antagonists displayed significant mosquito repellent activities with some of them causing anosmia-like effects to mosquitoes similar to equivalent doses of the widely used insect repellent DEET. Binary mixtures of the most active compounds caused stronger repellence effects relative to single compounds, a finding suggesting the possible existence of alternative binding sites on ORco and functional synergies caused by allosteric changes within the homomer. Antagonist competition assays against a known ORco agonist suggest the presence of different, orhosteric and allosteric binding sites for the antagonists on ORco and provide a mechanistic rationale for the enhanced repellent activities of the blends. These results demonstrate that natural ORco antagonists may modify olfaction-based mosquito behaviors in a predicted fashion. Moreover, the screening platform employed this study may yield enhanced possibilities for personal protection against mosquito-borne infectious diseases.

Exploring Conformational Dynamics of the Extracellular Venus Flytrap Domain of the $GABA_B$ Receptor: a Path-Metadynamics Study #10

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Researcher
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The $GABA_B$ receptor ($GABA_B$-R) is an obligate heterodimer comprised of $GABA_{B1a/b}$ and $GABA_{B2}$ that belongs to class C of metabotropic G-protein coupled receptors [1]. The main inhibitor neurotransmitter in the CNS, γ-amino-butyric-acid (GABA), binds to the orthosteric binding pocket of $GABA_B$-R which is located in an extracellular domain of $GABA_{B1a/b}$, called the Venus flytrap (VFT) [1]. Based on knowledge of other class C members, the $GABA_B$-R is presumed to undergo major structural rearrangements upon activation, starting from stabilization of the extracellular VFT in a closed/active conformation. Deficits in $GABA_B$-R signaling is associated with numerous neurological and neuropsychiatric disorders [2,3]. Gaining knowledge about molecular activation mechanism and druggable receptor conformations is crucial for understanding the receptor function and to guide rational drug design.
Unbiased molecular dynamics (MD) in combination with path-metadynamics was performed to gain mechanistic insights into the $GABA_B$ VFT conformational transition. MD simulations of six VFT X-ray structures representing both the open/inactive and closed/active states, all in the unbound form, were simulated for 1 µs. The sampled conformations were exploited to derive a path-based reaction coordinate describing the open/inactive-to-closed/active domain motion that was resampled through Well-Tempered metadynamics [4,5]. Our simulations confirm that the open and closed states are the main conformations adopted by the receptor and that they are separated by substantial energy barriers. Stable intermediate conformations were also identified, which might hold potential for future drug discovery efforts.

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Formation of a β2AR*-Gs_{GDP} intermediate complex

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Section:

- Macromolecular Interactions in Signalling Pathways,
- Biological Roles of Signal Transduction,
- Molecular Modulators of Signal Transduction,
- Advanced Methodologies and Technologies
- Public Web Resources

Position of the presenter: Postdoc
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X-ray crystallography and cryo-electron microscopy structures of receptor G protein complexes show hallmarks of receptor and G protein activation. In all these complexes, the C-terminal α5 helix of Gα binds deeply into the binding pocket of the activated receptor (R*). In contrast, recent hydrogen-deuterium exchange mass spectrometry and X-ray footprinting mass spectrometry data of the β2-adrenoceptor Gs system show that α5 remains dynamic for a long period of time suggesting an intermediate GDP-bound β2AR*-GsG_{DP} complex as the first specific receptor G protein interaction[1]. The existence of an intermediate R*-G_{GDP} complex was initially demonstrated by kinetic and single-molecule fluorescence resonance energy transfer analysis[2,3]. The recent X-ray structure of the β2AR construct with a fused Gαs carboxyl-terminal 14 amino acid peptide (PDB-id: 6E67) shows a binding mode of α5 that significantly differs from the X-ray structure of nucleotide-free β2AR*-Gs (PDB-id: 3SN6)[4]. In order to investigate whether that mode of interaction represents the β2AR*-Gs_{GDP} complex, we analyzed the spontaneous formation of the β2AR*-Gs_{GDP} complex in long all-atomistic classical molecular dynamics simulations. Results are discussed with regards to the role of structural intermediates for coupling specificity and for receptor catalyzed G protein nucleotide exchange.

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Dissecting the roles of GRK2 and GRK3 in μ-opioid receptor internalization and β- arrestin2 recruitment using CRISPR/Cas9-edited HEK293 cells

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Assistant Professor
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Most G protein-coupled receptors (GPCRs) recruit arrestins and are internalized upon agonist stimulation. For the μ-opioid receptor (μ-OR), this process has been linked to development of opioid tolerance. GPCR kinases (GRKs), particularly GRK2 and GRK3, have been shown to be important for μ-OR recruitment of arrestins and internalization. However, the contribution of GRK2 and GRK3 to arrestin recruitment and receptor internalization, remain to be determined in their complete absence. By CRISPR/Cas9 we established HEK293 cells with knock-out of GRK2, GRK3 or both to dissect their individual contributions in β-arrestin2 (arrestin3) recruitment and μ-OR internalization upon stimulation with four different agonists. We showed that GRK2/3 removal reduced agonist-induced μ-OR internalization substantially. Furthermore, we found GRK2 to be more important for μ-OR internalization than GRK3. In contrast, the effect of GRK2/3 knock-out on β-arrestin2 recruitment to the plasma membrane was minor. Rescue expression experiments restored GRK2/3 functions. The GRK2/3 small molecule inhibitor CMPD101 showed a high similarity between the genetic and pharmacological approaches, cross-validating the specificity of both. However, off-target effects were observed at high CMPD101 concentrations. These GRK2/3 KO cell lines should prove useful for a wide range of studies on GPCR function.

δ-branch of class A GPCRs: structural aspects of activation

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Postdoc

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Among five classes of G protein-coupled receptors, class A is the largest and the most studied. The mechanism of class A GPCR activation is generally established. Specifically, an agonist arriving from the extracellular space binds to the orthosteric pocket of its cognate receptor. Small conformational rearrangements in the ligand binding pocket trigger activation of conservative microswitches, leading to large-scale movements of transmembrane helices that open a cleft on the intracellular side of the receptor for the engagement and activation of G proteins and other transducers.[1] A key player in the class A GPCR activation mechanism is the sodium ion, that binds in the central part of the receptor, being coordinated by a highly conserved group of amino acids. Na^+ stabilizes the inactive state, and is presumably released into cytoplasm upon receptor activation.[2]

Based on sequence homology class A GPCRs is further divided into four branches, α-δ, which have unique structural and functional properties.[3] Delta-branch is the least clustered and the most distinct in terms of the receptor activation pattern. Recently we determined crystal structures of two δ-branch representatives: cysteinyl leukotriene receptors, $CysLT_1R$ and $CysLT_2R$,[4-5] which share ~30% identity. Both receptors in complexes with antagonists were captured in the inactive state, however, with an active-like conformation of the $P^{5.50}$-$I^{3.40}$-$F^{6.44}$ motif and TM7. In the $CysLT_1R$ structure, Na^+ is directly coordinated by four amino acids, that was not observed previously, however, in $CysLT_2R$ no Na^+ is present in the structure, which prompts us to further investigate the role of Na^+ in the activation mechanism of the δ-branch of class A GPCRs.

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The urotensin II G protein-coupled receptor relays key neurobiological mechanisms in subarachnoid hemorrhage through a Gq-dependent pathway

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Postdoc
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Cerebral vasospasm (CVS) is a severe complication of aneurysmal subarachnoid hemorrhage (SAH). Urotensin II (UII) is a vasoactive peptide activating the G protein-coupled receptor UT involved in vascular pathologies. We established that higher UII plasma level in SAH patients is predictive of CVS. Here we studied the role of the urotensinergic system in the occurrence of SAH-associated complications requiring major contribution of Gq instead of Gi pathways in a mouse model of SAH. To seek for the best therapeutic molecule, we evaluated the impact of two UT biased ligands mainly targeting the Gq pathway on *in vivo* consequences of SAH.
We used a double intracisternal blood injection SAH procedure in wild-type ($UT^{+/+}$), UT knock-down ($UT^{-/-}$) or humanized UT ($UT^{h+/h+}$) C57Bl/6 mice to investigate the impact of UT activation, the biased ligands, and the Gq- and/or Gi-signaling pathways on SAH-induced deficits.
In SAH $UT^{+/+}$ or $UT^{h+/h+}$ mice, UT appeared overexpressed in endothelial vasospasmed arteries, cortical capillaries and the meningeal compartment from day1 to day7 post-SAH. In $UT^{-/-}$ mice, SAH-evoked CVS, neuroinflammation, hippocampal apoptosis and cognitive dysfunctions observed in $UT^{+/+}$ and $UT^{h+/h+}$ were entirely absent. Intracisternal administration of the two biased ligands or Gq, PLC or PKC inhibitors totally prevented CVS and motor coordination impairment consecutive to SAH while Gi inhibition only partially attenuated SAH-associated complications.

This study demonstrate that UT partial antagonism prevents CVS and protect brain functions *via* inactivation of a Gq signaling pathway in the SAH, positioning UT as a key therapeutic target in SAH.

SESSION 4: GPCRdb / GPCRmd satellite meeting Saturday 28/03 15:45

Organizers: Jana Selent and David Gloriam

15:45 – 16:30 GPCRdb

Albert Kooistra & Christian Munk

GPCRdb (www.gpcrdb.org) contains data, diagrams and web tools for G protein-coupled receptors (GPCRs). Users can browse all GPCR structures and the largest collections of receptor mutants. Diagrams can be produced and downloaded to illustrate receptor residues (snake-plot and helix box diagrams) and relationships (phylogenetic trees). Reference (structure) structure-based sequence alignments take into account helix bulges and constrictions, display statistics of amino acid conservation and have been assigned generic residue numbering for equivalent residues in different receptors.

This demo will show some of GPCRdb's most used and new features:

- GPCRdb usage statistics
- Construct Design Tool
- Structure models
- Sequence Signature tool
- Mutation Data
- Residue function browser

16:30 – 17:15 GPCRmd

Jana Selent

GPCRmd (www.gpcrmd.org) is an online platform with web-based visualization capabilities and a comprehensive analysis toolbox that allows scientists from any discipline to visualize, inspect, and analyse GPCR molecular dynamics.

It is a community-driven initiative with the final aim to accelerate basic research and beyond the discovery of more efficient and safer therapeutics.

SESSION 5: Social Event

Saturday 28/03 18:00-20:00

We will meet up in five virtual rooms for casual chats among participants, organizers and speakers.

Join Breakout Rooms to find a group to mingle with. We can allow up to 100 participants to join by video and audio.

Here are some topics/groups you could join (*subject to last-minute changes*):

1. **Lockdown life** - Best strategies with kids, partners, and working from home
2. **Career & Opportunities** - Heard about a good grant? Collaborations and new strategies
3. **Meet the speakers** - join presenters and chairs of the conference
4. **WG1-3** Let's talk about science
5. **WG 4-5** Let's talk about science

Have a beer ready from your local brewery ↓, so we know from where you are joining us ☺

Biosensors reveal ligand- selective patterns of opioid receptor activation

Miriam Stoeber and Mark von Zastrow

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Rue Michel-Servet 1, 1211 Geneva, Switzerland

Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of a presenter: Principle Investigator / Group Leader
Email addresses of a presenter: miriam.stoeber@unige.ch

Opioid receptors (ORs) precisely modulate behaviour when activated by native peptide ligands but distort behaviours to produce pathology when activated by non-peptide drugs. A fundamental question is how opioid drugs differ from peptides in their actions on target cells. Here, we show that opioid drugs (1) differ in the subcellular location at which they activate ORs and (2) drive ORs to engage different cytoplasmic proteins. In our studies, we employ genetically encoded biosensors that directly detect agonist-induced activation of ORs and that are disconnected from endogenous transduction mechanisms. Using cell-based assays, we demonstrate differences between biosensor recruitment to ORs produced by chemically distinct opioid ligands. First, we establish an agonist-selective real-time map of the spatiotemporal organization of OR activation in living cells and then, we reveal agonist-selective recruitment of cytosolic proteins to activated ORs. The results establish an approach to probe the cellular basis of opioid drug action and reveal that agonists produce distinct receptor-based effects.

Systems analysis of G protein- coupled receptor pharmacology

Graham Ladds

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

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G protein-coupled receptors (GPCRs) remain one of the leading targets for therapeutic intervention. These cell surface receptors bind a plethora of different agonists leading to diverse intracellular signalling outcomes. For many years it had been incorrectly assumed that GPCRs bound a single agonist to elicit a response. However, there is now overwhelming evidence to suggest that, GPCRs exist in multiple receptor conformations. Biased agonism is the ability for a ligand to stabilise a certain GPCR conformation thereby directing its signalling to a distinct functional outcome. However, such information can make interpreting experimental data a challenge. Our approach to addressing these issues has been to combine computational biology and molecular pharmacology with a view to providing a systems-level understanding of G protein-coupled signalling. In this presentation I will describe our initial computational/experimental work modelling a GPCR signalling cascade in the simple eukaryotic organism yeast (Smith et al. 2009; Croft et al. 2013; Shaw et al. 2019) before describing how we have expanded the use of our models to described G protein-signalling bias in mammalian cells (Bridge et al. 2018).

The force within – Mechano-sensitive adhesion GPCR shape physiology

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Section:
Biological Roles of Signal Transduction

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Adhesion G protein-coupled receptors (aGPCR) are an enigmatic and highly versatile class of GPCR. Members of this receptor class are involved in many developmental processes, immune and synaptic functions. Their mode of action has been largely unknown but recent findings implicate the combination of external force application with subsequent exposure of the tethered agonist sequence as a prerequisite for aGPCR activation[1-3]. The administered mechanical force can either origin from the extracellular surrounding or from changes within the cell expressing the respective aGPCR. We have shown previously that the aGPCR GPR126 can be activated through the combination of vibration and interaction with Laminin211, which is the prerequisite to ensure proper myelination of axons[2]. Currently, we are focussing on the physiological function of the orphan aGPCR GPR133 using knock-out (KO) mice. We find that this mechano-sensitive receptor is essentially required to maintain normal heart rate and its impairment leads to dilated cardiomyopathy. This phenotype is already detectable in heterozygous animals, which is of high relevance as several natural variants of GPR133 have been identified in humans that inactivate the receptor[4], thus making this aGPCR a previously unrecognized cause of and potential drug target for the treatment of heart failure in human.

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Chemokine GPCRs function with different G proteins or anchoring partners to control glioma invasiveness

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

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Glioblastoma (GBMs) is one of most aggressive human cancers. Despite surgical resection followed by radio/chemotherapy, the prognostic of GBM patients is poor. The neurobiological nature of GBM cells leads to invasiveness of the brain parenchyma *via* chemotactic migration, sustaining at least in part resistance to treatments. To actively migrate within the brain, malignant glioma cells respond to chemical cues including chemokines, peptides and/or components of the extracellular matrix, harnessing micro-vascular and white matter routes.

In this context, we showed that chemotactic G protein-coupled receptors GPCRs CXCR4 and UT, and their respective ligands SDF-1α and UII, are highly expressed in GBM cells within hypoxic and perivascular areas exhibiting mesenchymal features. UT and CXCR4 receptors engaged pleiotropic Gαi and Gα12/13-mediating signalling pathways, leading to actin filament organization, lamellipodium expansion, and adhesion for invasion. However, the apparent redundancy of these chemokine receptors may at least in part, explain the failure of therapeutics targeting individual receptors.

The objective of our research is to understand how signalling nodes common to some GPCRs such as Gα, β and γ subunits or key anchoring proteins, control cell and glioma fates. We therefore highlighted variable levels of expression of *i)* the 31 subunits (15α, 5β and 11) and *ii)* Filamin A, a neurodevelopmental platform protein, using RNAseq data from large glioma patient cohorts, associated with poor prognosis and early recurrence. The way these signalling nodes drives the UT receptor activity was here investigated. It should open a new avenue to target GPCR-relayed mechanisms in various situations.

In silico and *in vitro* Approaches to Characterize a Novel G-Protein Coupled Receptor in *Thaumetopoea pityocampa*

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

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Insect neuropeptides regulate different aspects of the physiology of insects. They exert their function through binding to their cognate receptors, mostly G-protein Coupled Receptors [1]. Allatostatin neuropeptides as one of the most well-known neuropeptides are named as Insect Growth Regulators [2]. In this study, Allatostatin receptor type C (AstR-C) of *Thaumetopoea pityocampa*, a widespread pest in Mediterranean countries, was characterized for the first time combining *in silico* and *in vitro* studies with the final aim of designing small molecules capable of regulating the physiology of these pest organisms. Using RET-based techniques, the GPCR-specific characteristics of AstR-C such as the kinetics of G-protein recruitment and G-protein activation along with β-arrestin recruitment were investigated. Docking and molecular dynamics simulation approaches were conducted to predict the orthosteric pocket of the receptor which was further validated by FRET-based G-protein activation assay using the mutant AstR-C. As a result, it was found that binding of the native ligand at picomolar range to the receptor induces the $G_{\alpha i}$ subtype to be coupled to the receptor. β-arrestin, on the other hand, was found to be recruited at nanomolar range. Studies of the kinetics of G-protein recruitment and activation revealed that a brief stimulation of the receptor at nanomolar range is enough to obtain a long-lasting FRET response further emphasizing the potency of the native ligand (τ_{on}= 9.3 Sec and τ_{off}= 57.20 Sec and τ_{on}= 4.4 sec and τ_{off}= 64.69 Sec, respectively). G-protein activation assay showed the accuracy of *in silico* studies in identifying the binding pocket of AstR-C.

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SESSION 7: Working Group 3 (chair: Masha Niv & Fabrizio Fierro) Sunday 29/03 11:20

Tools and drugs for G proteins and G protein-coupled receptors

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1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

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For about 100 (non-olfactory) G protein-coupled receptors (GPCRs) the endogenous agonist still remains unknown or has not been confirmed. These so-called *orphan receptors* belong to the most enigmatic members of the GPCR superfamily. In order to study their roles and functions, and for validating them as (potential) novel drug targets, selective agonists and antagonists are required as tool compounds.
Our group has been interested in purinergic and lipid-activated GPCRs, including adenine, adenosine, nucleotide (P2Y), and cannabinoid receptors. Recently, we have extended our focus of interest to related orphan GPCRs belonging to the delta-branch of rhodopsin-like GPCRs, e.g. the nucleotide receptor-like GPCRs GPR17 and GPR35, and the lipid-activated GPCRs GPR18, GPR55 and GPR84.
Our goal has been to develop potent and selective ligands as biological tools that will allow pharmacological studies of these scarcely investigated orphan receptors. Our strategy includes preparation of stably expressing cell lines, development of suitable functional assays, screening of our compound library consisting of synthetic drug-like compounds and natural products, careful optimization of selected hit compounds by medicinal chemistry approaches including analysis of structure-activity relationships and homology modeling, and preparation of radioligands and fluorescent-labeled ligands, if feasible. Using this approach, we have been successful in developing novel potent and selective ligands for several orphan GPCRs (for recent examples see [1-4]).
GPCRs transduce their signals across cell membranes by activation of guanine nucleotide-binding proteins (G proteins). The mechanisms of signal transduction are currently not completely understood. Direct blockade of G protein subtypes, e.g. Gq proteins, has been proposed as a novel strategy for the treatment of complex diseases. We have recently developed the first radioligands for the sensitive detection of Gq proteins, which enable a variety of studies including the screening for novel G protein inhibitors [5].
Labeled tool compounds for GPCRs and G proteins are essential for the development and characterization of novel drugs.

(Supported by the Deutsche Forschungsgemeinschaft within FOR2372)

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SESSION 7: Working Group 3 (chair: Masha Niv & Fabrizio Fierro) Sunday 29/03 11:40

Ligand-directed affinity labelling – a new approach to visualise GPCRs

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Postdoc
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Fluorescent labelling of a protein of interest can be achieved in many different ways and is an absolute prerequisite for many of the techniques used to probe its function and localisation. For G protein-coupled receptors (GPCRs) this is often achieved through the use of fluorescent protein tags (e.g. GFP, SNAP tag), fluorescently labelled antibodies or fluorescent ligands. Each of these technologies has its limitations and are not readily transferrable to endogenously expressing systems where GPCRs are expressed at low levels. An orthogonal approach to protein labelling is through the use of ligand-directed chemistry whereby a fluorophore is connected via a highly reactive, electrophilic linker to a ligand that binds to the protein of interest and upon binding, this linker reacts with a nucleophilic amino acid side chain (Lys, Ser, Tyr) close to the binding site, forming a covalent bond between the fluorophore and protein. This then allows the ligand to dissociate from the binding site allowing the receptor to be stimulated or blocked with additional ligands as required.

Using the adenosine A_{2A} receptor ($A_{2A}R$) as a model system, we have developed ligand-guided labelling (LGL) compounds that can specifically covalently label the $A_{2A}R$ with a fluorophore. Through the use of ligand binding, functional and biochemical assays we have demonstrated that the LGL compounds covalently attach a fluorophore to the $A_{2A}R$. We have further shown that these LGL compounds can be used to study the $A_{2A}R$ in endogenously expressing systems. LGL is a novel, non-invasive approach to visualise and study GPCRs.

SESSION 7: Working Group 3 (chair: Masha Niv & Fabrizio Fierro) Sunday 29/03 12:00

Investigating functional selectivity in and around GPCRs with Molecular Dynamics

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1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

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The concept of functional selectivity refers to the possibility of modulating GPCR signaling, namely the interaction pattern of a receptor with its downstream effectors arrestin, G protein and GRKs, by changing their affinities or binding kinetics through specific GPCR ligands .
Exploring possible molecular determinants of functional selectivity of GPCRs entails both the structural and dynamical analysis of the receptor bound to different ligands, and on the opposite side the modulation of the effectors. Here two such complementary approaches are presented.
The first study focuses on the activation transition in the D2R starting by the inverse agonist bound crystal structure and comparing the effect of the endogenous ligand dopamine to the partial agonist aripiprazole and to an inverse agonist. Multiple microsecond Molecular Dynamics trajectories are analyzed: differences and similarities are highlighted by using orthogonal approaches, such as blind AI based methods and knowledge-driven dimensionality reduction and correlation analysis.

The second study focuses on the activation mechanism of arrestin, which helps this effector bind to the activated GPCR. We explore the possibility that the interdomain rotation coupled to arrestin activation and measured in MD simulations might be used to predict the intrinsic activation propensity of arrestin subtypes and mutants. Moreover we develop an in silico drug discovery approach to design small molecules to allosterically modulate the rotation propensity, to be used as chemical tools to enhance or inhibit binding.

SESSION 7: Working Group 3 (chair: Masha Niv & Fabrizio Fierro) Sunday 29/03 12:15

Design of a GPCR agonist by Targeting a Binding Site Water Network

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
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Solvent reorganization is a major driving force of protein-ligand association, but the contribution of binding site waters to ligand affinity is poorly understood. We investigated the effect of ligand interactions with a hydration network in the binding site of the A_{2A} adenosine receptor. Three analogs of the endogenous agonist adenosine, which either interacted with or displaced an ordered binding site water molecule, were studied experimentally and by molecular dynamics simulations. Chemical modification of the endogenous agonist adenosine that led to a loss of a hydrogen bond to the ordered water resulted in a loss of binding and this activity cliff was captured by molecular dynamics free energy calculations. Two compounds predicted to displace the ordered water from the binding site were then synthesized and evaluated experimentally, leading to the discovery of a novel A_{2A} agonist with nanomolar activity. Calculation of the thermodynamic profiles resulting from introducing substituents that interacted with or displaced a water revealed that the gain of binding affinity was driven by enthalpy. Detailed analysis of the energetics and binding site hydration networks revealed that the change in enthalpy was governed by contributions that are often neglected in structure-based drug optimization, which focuses primarily on receptor-ligand interactions. In particular, simulations suggested that displacement of water from a binding site to the bulk solvent can lead to large favorable contributions due to improved solvent-solvent interactions within the ligand biding site. These findings provide insights into the molecular driving forces of protein-ligand binding and novel strategies for rational drug design.

Computational approaches for the proinflammatory lipid-activated G protein-coupled receptor GPR84 providing structural insights

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1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction
4. Advanced Methodologies and Technologies
5. Public Web Resources

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The G_i protein-coupled receptor GPR84, which is activated by medium-chain (hydroxy)fatty acids, has attracted much attention due to its significant role in immunological functions.[1] 3,3'-Diindolylmethane and 6-octylaminouracil were identified as small molecule GPR84 agonists and the two class of compounds were significantly improved and their properties have been optimized.[2-4] Recently, a high-affinity agonist radioligand for GPR84, [^{3}H]PSB-1584 which binds to the putative orthosteric, fatty acid binding site was developed.[5] The radioligand was fully characterized and applied in competition binding assays. The determined binding data served as a basis for analyzing interactions of ligands with GPR84 studied by docking of compounds into a receptor homology model and by performing molecular dynamic simulation. The study reveals crucial elements of ligand binding and the predicted interactions of the compounds with amino acid residues in the binding pocket of the receptor were well in agreement with structure-activity relationships and reported mutagenesis data.[4-7] These results may eventually contribute to the rational design of pharmacological tools and drugs.

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Mechanisms Underlying Allosteric Molecular Switches of Metabotropic Glutamate Receptor 5 #11

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1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
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5. Public Web Resources

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Allosteric modulation of G protein-coupled receptors (GPCRs), and especially metabotropic glutamate (mGlu) receptors, has become an important strategy for drug discovery. Positive and negative allosteric modulators (PAM, NAM) are widely reported for the mGlu receptor family with leads mostly originating by high-throughput screening followed by iterative medicinal chemistry.[1] One of these, the mGlu5 receptor, shows an unexpected complete switch in functional activity despite only small changes in their chemical structure, resulting in PAM or NAM for the same scaffold.[2] Up to now the origins of this effect are not understood, causing difficulties in a drug discovery context. In this work, experimental data was gathered and analysed alongside docking and Molecular Dynamics (MD) calculations for three sets of PAM and NAM pairs. The results consistently show the role of specific interactions formed between ligand substituents and amino acid sidechains that block or promote local movements associated with receptor activation. The work provides an explanation for how such small structural changes lead to remarkable differences in functional activity. Whilst this work can greatly help drug discovery programs avoid these switches, it also provides valuable insight into the mechanisms of class C GPCR allosteric activation. Furthermore, the approach shows the value of applying MD to understand functional activity in drug design programs, even for such close structural analogues.

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Conservative mutation of phosphosensors in arrestin-1 (K14R, K15R) influences receptor-binding modes. #12

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Section: Macromolecular Interactions in Signalling Pathways
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Arrestin proteins regulate the signalling of G protein-coupled receptors (GPCRs). Termination of GPCR signalling requires phosphorylation of the receptor C-terminus and subsequent arrestin binding, which disables further coupling of the receptor to the G protein. In order to be activated for receptor core binding and high-affinity complex formation, arrestin interacts with the phosphorylated receptor C-terminus in a low-affinity 'pre-complex'. This interaction displaces the auto-inhibitory C-tail of arrestin, which normally stabilizes arrestin in a basal inactive state via a network of buried charged residues (polar core) and other interactions within the arrestin N-domain [1, 2]. Despite high-resolution structural data on basal, pre-activated and GPCR-bound arrestin-1 [2, 3, 4], as well as detailed insights into arrestin activation by molecular dynamics simulations [5], the structural mechanism of receptor-mediated arrestin activation is still not fully understood.

In this study we examined the functional role of two key lysine residues in the arrestin-1 N-domain, K14 and K15, which interact with the arrestin C-tail in the basal state and bind key phosphorylated sites on the receptor C-terminus in the high-affinity complex [1, 6]. Interestingly, conservative mutation of these sites to arginine resulted in impaired interaction of arrestin-1 with certain functional forms of the GPCR rhodopsin (the photoreceptor from retinal rod cells). 'Pre-complex'-like interactions with dark-state phosphorylated rhodopsin (RhoP) and phosphorylated aporeceptor opsin (OpsP) were hampered by the K14R,K15R mutations, while binding to light-activated phosphorylated rhodopsin (Rho*P) was not affected by the mutations. These observations were made using multiple techniques to observe and quantify arrestin-1 binding to rhodopsin, including centrifugal pull-down analysis, site-directed fluorescence spectroscopy, and UV-Vis absorbance spectroscopy. Flexible docking analysis of the arrestin-3 equivalent mutant (K11R, K12R) suggest that these mutations stabilize the arrestin C-tail more than the phosphorylated Rho C-tail and thereby favour an overall basal state of arrestin.

In conclusion, we hypothesise that the K14R, K15R mutations impede arrestin C-tail release and interaction with the receptor C-terminus in the pre-complex. Our results further suggest that additional contribution of the active receptor core upon Rho*P binding allows the mutant to be fully activated and engage in the high affinity interaction.

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Combination of pharmacophore fingerprint and artificial intelligence as a new approach in search for GPCR ligands #13

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

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Pharmacophores and fingerprints as separate instances are commonly used in computer-aided drug design. Both methods seem to be incompatible: pharmacophores are understood as steric orientation of features responsible for interacting with the protein and triggering biological effect, whereas fingerprint is structure representation in a form of bit string. Nevertheless, the first attempt of combining both approaches into the form of pharmacophore fingerprint was described by Gobbi and Poppinger in 1998 [1].
In this study original idea was extended for covering all possible combinations of pharmacophore patterns resulting in PharmPrint which contains 35k bits. This representation was applied as input data for machine learning approaches (SVC, linear SVC, logistic regression and neural networks) for the development of models able for separating actives from in actives which may be used at the initial steps of virtual screening cascades.
Length and resolution of PharmPrint enforced testing and application of several reduction algorithms which decrease time-consumption without simultaneous decreasing of statistical parameters. Testing and optimization of several machine learning methodologies, selection of the best representation of the input data (all data were fetched from ChEMBL database) allow for the development of a new methodology which outperformed classical fingerprints (CDK, Estate, MACCS, Substructure, Klekotha-Roth, etc.) in separating GPCR ligands and can be applied as an alternative for classical pharmacophore modeling and 2D fingerprintins.
The whole module will be available on-line as a part of web tool for computer-aided drug design of compounds with the polypharmacology profile.

Acknowledgments
The work was supported was supported by the Polish National Centre for Research and Development grant LIDER/37/0137/L-9/17/NCBR/2018

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SNatural, a natural product cheminformatics database for virtual screening on transmembrane proteins #14

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

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Natural products are chemical compounds found in nature originating in plants, animals, and microbial and marine sources. In this work, we have gathered chemical information of ~100k naturally occurring compounds. Furthermore, several chemical descriptors (~30) have been calculated, such as membrane permeability, molecular weight, AlogP, hydrogen bond acceptors and donors and aromaticity, to further fine-tune the selection of natural compounds towards virtual screening efforts. The database has been compiled in an SQL platform for rapid search and selection of subsets related to the receptor to be targeted, towards the identification of potential natural bioactive compounds.

Acknowledgment
This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (T1EDK-01921)

Study of Mechanism of APP Proteolysis by the Gamma-Secretase Complex #15

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: PhD student
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Alzheimer's disease is the most common progressive neurodegenerative disorder and is characterized by the presence of amyloid β (Aβ) plaques and neurofibrillary tangles in the brain. No treatments are yet available to cure Alzheimer's disease, however, soluble Aβ oligomers are believed to play a crucial role in the neuroinflammation that is observed in this disease. The γ-secretase complex, which produces Aβ, consists of the catalytic subunit presenilin which is associated in a 1:1:1:1 stoichiometry with three subunits: PEN-2, APH-1, and nicastrin (NCT). The γ-secretase is an intramembrane-cleaving protease involved in many physiological processes. Their clinical relevance comes from their involvement in Alzheimer's disease, cancer, and other disorders. The clinical trials with the γ-secretase inhibitors have, however, demonstrated that unselective inhibition of γ-secretase causes serious toxicity. Evolving insights suggest that more subtle modulations of γ-secretase proteolysis are potentially valuable approaches but details of substrate-enzyme interactions needs to be revealed first. In our study we refined the cryo-electron microscopy (cryo-EM) structure of γ-secretase complex, investigate binding and partial unfolding of fragments of amyloid precursor protein (APP) in the binding site of presenilin, to uncover influence of conformational dynamics of substrate and the enzyme on mechanism of sequential cleavage of APP.

Conformational thermostabilisation of GPCRs for structure-based drug design #16

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1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

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G protein-coupled receptors (GPCRs) represent the largest family of druggable targets in the human genome. Despite being one of the most important classes of proteins for drug discovery, the design of drugs targeting GPCRs remains challenging due to their inherent flexibility and instability outside of the membrane. Sosei Heptares' StaR® platform technology overcomes these challenges through protein engineering. By identifying a minimal number of thermostabilising mutations, a receptor can be locked in a specified conformation that enhances its survival in a detergent environment. This approach has been applied to GPCRs from families A, B and C, using peptide or small molecule ligands, and to orphan receptors with no known ligands. The resulting stabilised receptors (StaR® proteins) are more amenable to purification, crystallisation and biophysical analysis of ligand binding.

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Extracellular Lactate-Mediated Increase in cAMP Enhances Aerobic Glycolysis in Astrocytes #17

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Assoc. Prof. / Group Leader
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In brain L-lactate can act as neuronal fuel and as a signal. Whether extracellular L-lactate affects brain metabolism, in particular metabolism of astrocytes, neuroglial cells, which produce L-lactate in aerobic glycolysis and release it through plasma membrane lactate transporters, is unclear. Recent studies suggested that astrocytes express low levels of the L-lactate GPR81 receptor ($EC_{50} \approx 5$ mM). In adipocytes GPR81 is part of an autocrine loop, in which the G_i-protein mediates reduction of cytosolic cyclic adenosine monophosphate (cAMP). To study whether a similar signaling mechanism is present in astrocytes, affecting aerobic glycolysis, we measured the cytosolic levels of cAMP, D-glucose, and L-lactate in astrocytes at a single cell resolution using fluorescence resonance energy transfer (FRET)-based nanosensors. In contrast to the situation in adipocytes, in astrocytes stimulation by extracellular L-lactate and the selective GPR81 agonists, 3-chloro-5-hydroxybenzoic acid (3Cl-5OH-BA), like adrenergic stimulation, elevated intracellular cAMP and L-lactate, which was reduced by the inhibition of adenylate cyclase. 3Cl-5OH-BA increased cAMP also in GPR81-knock out astrocytes. This indicates that the observed effect is GPR81-independent and likely mediated by a novel unidentified excitatory L-lactate receptor in astrocytes that enhances aerobic glycolysis and L-lactate production by a positive feedback mechanism.

HomolWat: a web server tool to incorporate "homologous" water molecules into GPCR structures #18

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Principal investigator
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Internal water molecules play an essential role in the structure and function of membrane proteins including G protein-coupled receptors (GPCRs). However, technical limitations severely influence the number and certainty of solved water molecules in 3D structures. This brings packing defects in the structures that may compromise further structural studies such as docking calculations or molecular dynamics simulations. Usable tools for modeling water molecules are sparse. Here we present HomolWat, a web application incorporating water molecules into protein structures by using template-based modeling of homologous water molecules obtained from high-resolution structures. The input is a 3D structure file in Protein Data Bank format whereas the output is the same structure with all homologous internal water molecules. This hydrated structure can be visualized interactively using NGL viewer [1] and downloaded for further use.
HomolWat has been successfully applied, as part of the pipeline used in the GPCRmd project (http://gpcrmd.org), the first open, interactive, and standardized database of GPCR molecular dynamics simulations [2]. While there are various tools available to predict the positions of internal waters using energy-based methods [3], the approach of borrowing lacking water molecules from homologous structures makes HomolWat unique. The web server is freely available at https://lmc.uab.es/homolwat and is currently focused on GPCRs. Extension to other membrane protein families is underway.

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Biased agonism at adenosine A2A receptor #19

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: PhD student
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Biased agonism, the ability of agonists to differentially activate downstream signalling pathways by stabilizing specific receptor conformations, is a key issue for G-Protein Coupled Receptors(GPCRs)[1]. An interesting case study for such phenomenon could be adenosine A_{2A} receptor, as it is one of the most relevant drug targets within the GPCR superfamily, and it gathers a vast amount of information[2]. For instance, it is the GPCR that accumulates the highest number of reported structures (49), including inactive, intermediate and active conformations.[3] This availability of resources allows for the exploration of the effects of certain agonists on the behaviour of the receptor. Taking advantage of this fact, and in combination with experimental results, the aim of our study is to explore how can 5 paradigmatic agonists (adenosine, NECA, CGS- 21680, PSB-0777 and LUF-5843) module the receptor in distinct ways, so as to trigger distinct signalling pathways. Both unbiased molecular dynamics simulations and signalling assays are put together in an attempt to provide insights on the molecular mechanisms underlying biased agonism on GPCRs.

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SESSION 12: Working Group 4

(chair: Nuska Tschammer & Eddy Sotelo) Monday 30/03 9:00

Watching receptors at work – using the microscope to study GPCRs

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction
4. Advanced Methodologies and Technologies
5. Public Web Resources

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SESSION 12: Working Group 4

(chair: Nuska Tschammer & Eddy Sotelo) Monday 30/03 9:50

Gene expression signatures - footprints of GPCR signalling pathway activity

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: Primary Investigator
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Inferring signalling pathway activities from high-throughput measurements is a key question of current systems biology research. The most frequently used data type for these kinds of analysis is gene expression data (microarray, RNAseq). Classical pathway analysis methods use the expression changes of pathway member genes to predict pathway activities. Recent “footprint” based methods, using the gene expression changes of pathway regulated genes, allow a more efficient way to analyse signalling pathway activities. Large scale perturbation gene expression datasets (like LINCS-L1000, with more than 1,000,000 gene expression profiles) can further advance the usability of gene expression footprint based methods, and can help to identify compounds with specific effects on selected pathways. With the recent advancements of single cell RNAseq methods, these methods can also help to better understand receptor - ligand interactions regulating intercellular communication.
After a short introduction to gene expression footprint based pathway activity inference methods, their usability to analyse GPCR signalling will be discussed.

SESSION 12: Working Group 4

(chair: Nuska Tschammer & Eddy Sotelo) Monday 30/03 10:10

Exploration of allosteric communication networks that underlie GPCR functionality

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2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction
4. Advanced Methodologies and Technologies
5. Public Web Resources

Position of the presenter: PhD student
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G-protein coupled receptors (GPCR) are highly flexible signaling machines with the ability to adopt diverse conformational states that are linked to distinct intracellular signaling responses. The transition between those states is mediated by a network of dynamically correlated residues called allosteric communication network (ACN). Molecular dynamics simulations have been extensively used to characterize ACNs due to their high spatial and temporal resolution. Current approaches use primarily one single descriptor like inter-residue contact1 and residue position correlation2. However, such representation does not capture all details of allosteric communication in GPCRs. To address this gap, we are currently developing a novel pipeline to study ACN by combining the advantages of different methods (e.g. rigid residue scan, anisotropic thermal diffusion, movement correlation, etc.). Here, we showcase the use of angle correlation as a useful parameter to describe receptor inactive and active states. Yet, capturing the differences between signaling states that are structural highly similar (e.g. Gs versus Gi-coupled) will require a more complete description of allosteric communication within the receptor (i.e. use of more descriptors and their relationships). We envisage that such an approach can help to increase the power of the analysis for ACNs and protein dynamics in the field of GPCRs and beyond. Ultimately, a better understanding of the complexity of ACNs in GPCRs can also open new avenues to improved drug development strategies.

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SESSION 12: Working Group 4

(chair: Nuska Tschammer & Eddy Sotelo) Monday 30/03 10:25

Novel fluorescence ligands for the characterization of muscarinic acetylcholine receptors

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies,
5. Public Web Resources

Position of the presenters: PhD students
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Muscarinic acetylcholine receptors (mAChR) are pharmacologically important class of GPCRs which have crucial regulatory role in both the central nervous system and the heart. mAChRs are also linked to several severe diseases such as Alzheimer´s and Parkinson´s disease [1]. The characterization of GPCR by the fluorescence methods have nowerdays become one of the most useful approaches. However, for mAChRs only a limited number of fluorescence ligands are available [2], and show no M2 selectivity. We have implemented two novel TAMRA labelled dibensodiazepinone derivates CG72 and MK342, for characterization of three different mAChR receptor subtypes (M1,2,4). Both of these labelled ligands could be used for ligand binding studies to mAChR subtypes in budded baculoviruses using fluorescence anisotropy assay. Furthermore, both fluorescence ligands show selectivity for M2 subtype compared to M1 and M4. CG72 shows quite fast and reversible binding to all studied subtypes proving it to be a good tool for screening other unlabelled ligands. In contrast MK342 shows slow and partially irreversible binding to M2 subtype, which makes it an interesting compound for studying tissue preparations.

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hGPCRnet: A tool for Cell-Type Specific Analysis of GPCR Signaling Pathways

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

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The G protein-coupled receptor database, GPCRdb

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3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

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The GPCR database, GPCRdb comprises reference data, analysis tools, interactive visualisation and experiment design tools (www.gpcrdb.org)[1].
GPCRdb sections that will be presented (U=unpublished):

1. **GPCR structures and models:** models of inactive, intermediate and active states – except for classes C and F that only have inactive templates[1].
2. **[U]G protein** resource: GPCR couplings and structure interactions & models. This will expand the resource from a previous study on GPCR-G protein selectivity[2].
3. **[U]Biased ligand and pathway-function** databases (data from articles and patents).

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GPCRmd: Bringing transparency and GPCR MD data within reach

Mariona Torrens-Fontanals[1], Ismael Rodríguez-Espigares[1] , Johanna K.S. Tiemann[2] , GPCRmd community[3] , Ramon Guixà-González[4,5] and Jana Selent[1]

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The functionality of G protein-coupled receptors (GPCRs) is highly determined by their ability to transition between distinct conformations. Because of this, understanding the structural dynamics of these receptors is essential for advancing our knowledge on their physiology. Molecular dynamics (MD) simulations is a well-established method for the characterization of such motions at an atomic level. However, the analysis and visualization of MD simulations require efficient storage resources and specialized software, limiting the dissemination of this data to specialists in the field.

GPCRmd[1] is an online resource that aims to approach the valuable information of MD simulations to all researchers interested in GPCRs, independently of their background. As such, GPCRmd provides free access to a vast number of GPCR MD simulations, being the first open-access research resource hosting MD simulations of most GPCR crystal structures solved to date. Moreover, GPCRmd simplifies the analysis of the simulation data. It is equipped with a comprehensive set of intuitive online tools for the interactive visualization and analysis of the MD simulations. Such tools allow, for example, the study of protein motions involving conserved, pharmacologically relevant, or diseased-related residues.

Here, we present the main features of GPCRmd, focusing on recent updates, and discuss the next steps of this project. Our final aim is to provide a resource containing MD data of all GPCRs with known structure, together with a complete set of visualization and analysis tools that covers the needs of the community of GPCR scientists.

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Membrane domain localization and mobility of G_s signaling cascade components

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3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
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Spatial segregation of G protein signaling cascade components and temporal dynamics of signaling remain insufficiently understood. We set out to determine spatial distribution and mobility of G_s signaling components in clathrin-coated structures (CCSs) and caveolae at very low expression levels. To achieve this goal, we used total internal reflection fluorescence microscopy (TIRF) and single-molecule imaging. Our results show that activated β_2-adrenergic receptor and arrestin 3 accumulate in CCSs similarly at high and very low expression levels, which is accompanied by a pronounced decrease in their mobility. Activated G_s protein subunits also exhibit reduced mobility but don't show accumulation in or exclusion from CCSs. None of the studied signaling proteins, except adenylyl cyclase 5, show detectable accumulation in caveolae. We conclude that the probability of efficient simultaneous clathrin-mediated endocytosis of β_2-adrenergic receptor, G_s protein and adenylyl cyclase 5 is low.

Dynamic pharmacophores unveil binding mode ensembles of partial GPCR agonists

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Section

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
5. Public Web Resources

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Given the essential role of G protein coupled receptors (GPCRs) in many (patho)physiological processes, more than 30 % of currently marketed drugs deploy their therapeutic effect by targeting GPCRs. However, the molecular understanding of how ligands translate their chemically encoded information to intracellular signalling is still incomplete, which renders novel methodologies for studying GPCRs highly necessary for rational drug design [1,2].

Here, we will focus on a novel computational approach, which combines classical three-dimensional pharmacophores with MD-based sampling in a fully automated fashion. The resulting dynamic pharmacophores (dynophores) provide information about the interaction pattern in space and time [3,4]. This enables us to mechanistically understand protein-ligand complexes as dynamic entities.

We will demonstrate the descriptive power of dynamic pharmacophores on a challenging task in GPCR research, the mechanistic basis for partial receptor activation. Using muscarinic receptors as a model system we show the existence of binding mode ensembles for classical partial muscarinic agonists like pilocarpine or arecoline [5]. By a comparison with the full agonist iperoxo and the inverse agonist QNB, we can link distinct binding modes with receptor activation and deactivation.

Furthermore, we discuss the concept of binding mode ensembles in the context of crystallographic data and recent NMR studies, which indicate that binding mode ensembles might play an important, but underestimated role for ligand-dependent pharmacological effects at GPCRs and other drug target classes.

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Pharmacological approaches to decipher the signaling networks of GPCR of the reproductive axis

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Section

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction
4. Advanced Methodologies and Technologies
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Our general research project aims at deciphering the signaling mechanisms whereby G protein-coupled receptors of the reproductive axis act at the cell level. The emphasis is put on the FSHR and on the LHR. Although these receptors have been studied for several decades now, there are still important gaps in the basic knowledge of the mechanisms whereby they work in the cell. To date, these gaps preclude to comprehensively understanding how these mechanisms are integrated into an adapted biological response that is ultimately translated into the dynamics of cell populations (proliferation/ survival/ apoptosis) and into the production of steroids in tightly regulated time frames during the ovarian cycle or spermatogenesis.

To decipher the dynamics of the signaling networks induced by the FSHR and LHR, we build mathematical models in order to quantitatively assess the role of each signaling protein within these networks. We measure signaling events in real time to constraints mathematical models[1,2,3,4] and to be able to make predictions. In addition, to understand better the dynamics of these signaling systems, or to test new hypotheses, we disturb these dynamics using FSHR/ LHR biased ligands. The latter are small antibody fragments (V_HH) that behave as modulators of receptor activity, with intrinsic biochemical features precious to explore the whole pharmacological spectrum of the FSHR and LHR dynamic changes. Beyond their interesting properties for basic science, these ligands herald potential applications to control reproduction in male and female, not only to improve methods of medically-assisted reproduction and substitute proteins to steroids for human or pet contraception, but also to synchronize female ovulation for artificial insemination in the agronomic field.

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Hydroxy-carboxylic acid receptor 2 signalling at endosomal compartments

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Section:

1. Macromolecular Interactions in Signalling Pathways,
2. Biological Roles of Signal Transduction,
3. Molecular Modulators of Signal Transduction,
4. Advanced Methodologies and Technologies
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Hydroxy-carboxylic acid receptor 2 (HCA2) is highly expressed in adipose tissue, where it has been shown to maintain metabolic homeostasis under changing metabolic and dietary conditions. The classical view that G protein-coupled receptors (GPCR) signalling occurs exclusively at the cellular membrane has been challenged by the discovery that GPCRs can signal via G proteins at intracellular sites.[1,2] Previous studies suggest that subcellular confinement of signalling events downstream of GPCR activation − e.g., cyclic adenosine monophosphate (cAMP) production and protein kinase A (PKA) activation – influence adipocyte development and lipolysis.[3] However, it is unknown whether HCA2 signals at intracellular sites and whether this accounts for HCA2 antilipolytic effects.

To investigate HCA2 signalling at intracellular sites, a BRET-based assay was used to quantitatively monitor, in living cells and in real-time, the recruitment of G proteins to subcellular compartments. Our results indicate that, HCA2 is predominantly coupled to $G\alpha_i$ and efficiently internalises to early endosomes upon stimulation with the full agonist nicotinic acid (NA). Additionally, following stimulation with NA, HCA2 recruits mini $G\alpha_i$ protein probes to membranes of early endosomes and possibly other endosomal compartments, indicating that HCA2 remains active following internalisation. Interestingly, a cell-impermeable HCA2 agonist (monomethyl fumarate), was also found to mediate $G\alpha_i$ signalling following receptor internalisation, albeit to a lesser extent (approximately 30% of the response to NA). This suggests that an intracellular pool of HCA2 may contribute to the response to NA.

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SESSION 15: Meeting Closure (chair: Martha Sommer) Monday 30/03 14:00

Dear Participants,

Thank you for joining our first online meeting. We hope you enjoyed the experience, as both inspiration for your work and distraction from the worrying daily news.

As this is our first attempt at an online conference, we are curious to 'see how it goes'. We may choose to hold future ERNEST meetings in this format.
Please share your comments or thoughts about the online conference experience by emailing ernest.ca18133@gmail.com.

As Chair of ERNEST, I am amazed at how quickly our Action has grown into a diverse, enthusiastic, and tightly connected community. I thank each of you for your participation and involvement!

In these uncertain times, it is more important than ever that we STAY CONNECTED.
Not only to keep up with collaborative research, but to SUPPORT EACH OTHER.

Take care, and best of health to you and your families,

Martha

Dr. Martha E. Sommer
Chair of your COST Action CA18133 ERNEST