GLISTEN Budapest 2014 Conference

Work group meeting

Training School

GPCRDB Satellite Meeting

October 2-4, 2014

Budapest, Hungary

EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

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Research Centre for Natural Sciences

Hungarian Academy of Sciences

Abstract booklet

Useful information

Scientific committee:

Peter Kolb - Philipps-University Marburg, Pharmaceutical Chemistry, Marburg, Germany

Chris de Graaf - VU University, Medicinal Chemistry, Amsterdam, Netherlands

György Keserű - Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

Thematic divisions:

Working Group 1: Dynamics - Xavier Deupi (CH) and Jan Steyaert (BE)

Working Group 2: Biased Signalling - Hans Bräuner-Osborne (DK) and Martine Smit (NL)

Working Group 3: GPCR Lipid/Protein Interactions - Masha Niv (IL) and Jana Selent (ES)

Working Group 4: Discovery & Design of GPCR Ligands - Esther Kellenberger (FR) and György Keserű (HU)

Local organizers:

György Ferenczy, György Keserű, Dávid Bajusz, Ádám Kelemen and Rita Csilla Tóth
Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

Márton Vass and Ákos Tarcsay
Gedeon Richter Plc, Budapest, Hungary

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We wish to express our gratitude to you for your contribution.

Web page:

http://glisten.ttk.mta.hu/

Venue:

The GLISTEN Budapest 2014 Conference will be held in the main conference room of the Research Centre for Natural Sciences.

Location:
1117 Budapest,
Magyar tudósok körútja 2.

Programme

October 2, Thursday

08:30-09:00	Registration of participants	
09:00-09:30	Peter Kolb (GLISTEN Chair)	
	Update about recent developments and plans	

Session I. Working Group 3

Chairs: Mickey Kosloff and Iro Georgoussi

09:30-10:15	Matthew Todd (University of Sydney, UK)	**LP01**
	Open source approaches to discovery in medicinal chemistry	
10:15-10:45	Zafiroula Georgoussi (National Centre for Scientific Research, GR)	**L301**
	Opioid receptor signaling mechanisms: beyond the G-Protein paradigm	
10:45-11:15	Coffee break	
11:15-11:45	Mickey Kosloff (University of Haifa, IL)	**L302**
	Deciphering and re-designing interaction specificity in G-protein signaling	
11:45-12:00	Martha E. Sommer (Charité - Universitätsmedizin Berlin, DE)	**L303**
	Formation of the arrestin-GPCR Complex: A tale of two loops	
12:00-12:15	Rosana Inácio dos Reis (Oxford University, UK)	**L304**
	Formation, purification and characterization of a pharmacologically relevant and functionally competent β2 adrenergic receptor-β arrestin complex	
12:15-12:30	Stefan Mordalski (Institute of Pharmacology PAS, PL)	**L305**
	Computational study on GPCR – G protein interfaces	
12:30-12:45	Chayne Piscitelli (Paul Scherrer Institute, CH)	**L306**
	Structural studies of GPCRs	
12:45-13:00	Patricia M. Dijkman (University of Oxford, UK)	**L307**
	Dynamic GPCR dimerization shown by ensemble and single molecule FRET, and DEER	
13:00-14:00	**Lunch and poster session**	
14:00-16:00	***MC meeting***	
14:00-15:20	***Training school***	
	GPCR pharmacology and fragment screening on GPCRs	
	András Visegrády (Gedeon Richter Plc., Budapest, HU)	
	Rob Leurs (VU University Amsterdam, NL)	

Session II: Working Group 2

Chair: Marc Parmentier

16:00-16:45	Rob Leurs (VU University Amsterdam, NL)	**L201**
	Mapping the structural determinants of histamine receptor binding kinetics	

16:45-17:20	Daniel Fourmy (University of Toulouse, FR) *Characterization of internalization of the human GIP receptor enables discovery of a biased agonist*	**L202**
17:20-17:50	Coffee break	
17:50-18:25	Jean-Yves Springael (Université Libre de Bruxelles, BE) *Biased signaling at chemokine receptors does not follow the G-protein versus ß-arrestin paradigm*	**L203**
18:25-19:00	Dietmar Weichert (Friedrich-Alexander University Erlangen-Nuremberg, DE) *Probing a molecular determinant for biased agonism at the ß2-adrenoceptor*	**L204**
20:00	**Evening activity:** Thermal bath (Rudas Fürdő) Location: Budapest, Döbrentei tér 9.	

October 3, Friday

Session III. Working Group 4

Chairs: Esther Kellenberger and György Keserű

09:00-09:45	Jan Steyaert (Vrije Universiteit Brussel, BE)	**LP02**
	Nanobody-enabled fragment screening on active-state constrained GPCRs	
09:45-10:15	Holger Stark (Heinrich-Heine-Universitaet, DE)	**L401**
	Novel agonists for the dopamine D3 receptor subtype with high In vivo activity (WG4)	
10:15-10:45	Dominique Bonnet (University of Strasbourg, FR)	**L402**
	Fluorescent probes to track GPCR binding and dimerization (WG4)	
10:45-11:20	Coffee break	
11:20-11:30	Katarzyna Kieć-Kononowicz (Jagiellonian University, PL)	**L403**
	Annelated xanthines as A1/A2A/A2B adenosine receptors ligands	
11:30-11:40	Annika Kreuchwig (Leibniz-Institut für Molekular Pharmakologie (FMP), DE)	**L404**
	Novel release of the GPCR-SSFE platform for automatic multiple-template based GPCR homology modeling	
11:40-11:50	Jhonny Azuaje (University of Santiago de Compostela, ES)	**L405**
	UGI-based engineering provides convergent assembly and novel architectures for homobivalent Ligands	
11:50-12:00	Juan Carlos Mobarec (University of Marburg, DE)	**L406**
	In-silico targeting of obestity: The case of the human bile acid receptor	
12:00-12:10	Marie Chabbert (CNRS - INSERM, FR)	**L407**
	Evolutionary hubs in selected GPCR families	
12:10-12:40	Esther Kellenberger (Université de Strasbourg, FR)	**L408**
	GLISTENDB presentation	
12:40-14:30	**Lunch and poster session**	
13:30-14:30	***Public GPCRDB demo of new features***	

Session IV. Working Group 1

Chairs: Xavier Deupi and Jan Steyaert

14:30-15:15	Francesca Magnani (University of Pavia, IT)	**L101**
	Tuning of ligand selectivity to GPCR subtypes by electronic sculpting	
15:15-16:00	Dmitry Veprintsev (Paul Scherrer Institut and ETH Zurich, CH)	**L102**
	Protein Backbone NMR Reveals Ligand Recognition, Signal Transmission, and quenching of motions by thermostabilization in the β1-adrenergic receptor	
16:00-16:30	Coffee break	
16:30-16:50	Bruck Taddese (CNRS-French National Centre for Scientific *Research, FR)*	**L103**
	Correlated motions in distinctive conformational states of the chemokine receptor CXCR4	

16:50-17:10 Luc Veya (EPFL, CH) **L103**

Single-molecule microscopy deciphers the relation between trafficking and signaling of the NK1 receptor in living cells (WG1)

17:10-17:30 Mauricio Esguerra (Uppsala University, SE) **L105**

New features for GPCR modeling implemented on GPCR-MODSYM and PYMEMDYN

17:30-19:30 ***Training school***

Fragment docking and virtual screening on GPCRs

Márton Vass (Gedeon Richter Plc., Budapest, HU)

Chris de Graaf (VU University Amsterdam, NL)

20:00 **Conference dinner**

The dinner will take place on a boat. The boat departs from a port near the conference venue.

October 4, Saturday

09:00-13:00 ***GPCRDB Satellite Meeting*** (parallel with Training school)

09:00-11:00	*Introduction and server/mutagenesis committees*
	David Gloriam (University of Copenhagen, DK)
11:00-11:30	Coffee break
11:30-13:00	*Joint discussion, applications & publications*
	David Gloriam (University of Copenhagen, DK)

09:00-13:00 ***Training school***

09:00-11:00	*Fragment optimizations*
	Greg Makara (ComInnex Budapest, HU)
	György Ferenczy (RCNS-HAS Budapest, HU)
11:00-11:30	Coffee break
11:30-13:00	*Synthesis lectures*
	Tibor Soós (RCNS-HAS Budapest, HU)

13:00-14:30 Lunch

14:30-18:00 ***Training school***

14:30-16:00	*Retrosynthesis*
	Maikel Wijtmans (VU University Amsterdam, NL)
16:00-16:30	Coffee break
16:30-18:00	*Reaxys tutorial*
	Maikel Wijtmans (VU University Amsterdam, NL)

Lecture abstracts

Numbering: Lxyy, L-lecture, x- Working Group number or P for plenary, yy- sequential number

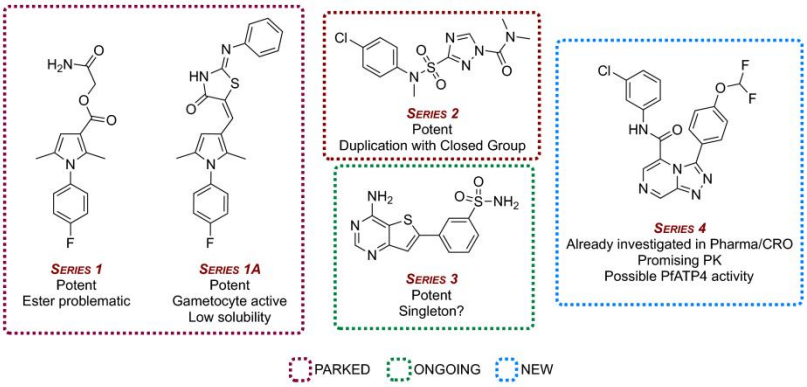
LP01

OPEN SOURCE APPROACHES TO DISCOVERY IN MEDICINAL CHEMISTRY

Matthew H. Todd[1]

1. *School of Chemistry, The University of Sydney, NSW 2006, Australia and the Open Source Malaria Consortium*

An *Open Source* approach to science involves the complete sharing of data and ideas, and the possibility that anyone may participate. The approach is therefore distinct from one described as *open innovation*, in which there is no requirement to reveal data or progress to others. While patents underpin open innovation, they are typically not sought in open source. My laboratory has applied open source principles to drug discovery and development in two areas relevant to tropical diseases. The first project, run in collaboration with the World Health Organisation, discovered a new route to the active enantiomer of praziquantel, the drug of choice for the hundreds of millions of people at risk of schistosomiasis.[1][2] The second project, in collaboration with the Medicines for Malaria Venture performs hit to lead/lead optimisation projects based on small molecule compounds identified and released by the pharmaceutical industry.[3][4] This talk will highlight the challenges and benefits of such an approach, and show that they have been built with tools that are publicly available or open source; thus any community of researchers can collaborate in a similar way. In the case of drug discovery, the loss is economic clarity around the end point of the development process. The gains include inputs to the project from the wider community, transparency, reproducibility, connections to the patient community and the project's ability to adapt quickly to changes in resources.



References:

1.) Woelfle, M., Olliaro, P., Todd, M. H., Nature Chemistry, **2011**, *3*, 745-748. DOI: 10.1038/nchem.1149

2.) Woelfle, M. et al., PloS Negl. Trop. Dis., **2011**, *5(9)*, e1260. DOI: 10.1371/journal.pntd.0001260

3.) Robertson, M. et al., Parasitology, **2014**, *141*, 148-157. DOI: 10.1017/S0031182013001121

4.) http://opensourcemalaria.org/

L301

OPIOID RECEPTOR SIGNALING MECHANISMS: BEYOND THE G PROTEIN PARADIGM

Zafiroula Georgoussi

Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", 15310 Ag. Paraskevi, Athens, Greece

Evidence has demonstrated that G protein coupled receptors (GPCRs) can physically interact with a variety of accessory proteins, confirming that signal transduction of these receptors is not restricted to heterotrimeric G protein activation. Such interactions can alter the effectiveness of agonist-driven cell signalling, determine the signals generated and alter the trafficking, targeting, fine tuning and cellular localization of these receptors by providing a scaffold that links the receptors to the cytoskeletal network (Georgoussi et al., 2013). Opioid receptors which belong to GPCRs modulate a variety of physiological responses in the nervous system through activation of a diverse array of effector systems ranging from adenylyl cyclase, ion channels to other signalling intermediates. Opioid administration causes activation of several transcription factors, including CREB, NF-kB, STAT3 and STAT5A/5B (Tso and Wong 2003, Mazarakou and Georgoussi, 2005; Georganta et al., 2010). Such parallel manifestations suggest that these receptors are involved in different signaling circuits that lead to alterations in target gene expression in a pleiotropic fashion. In the present study emphasis will be given to unconventional interacting partners of the μ- and δ-opioid receptors such as the Regulators of G protein signalling (RGS) proteins and STAT5B. Evidence will be presented on which RGS proteins opioid receptors interact; how RGS4 and RGS2 confer selectivity to the receptors to choose a specific subset of G proteins; how activation of opioid receptors result in recruitment of RGS proteins to the plasma membrane; and how RGS proteins exert a differential modulatory effect in opioid receptor signalling. Moreover evidence will be presented on how STAT5B associates with the δ-opioid receptor (δ-OR) and forms selective pairs with selective Gα and Gβγ subunits and RGS proteins; and how activation of the δ-OR with selective agonists promotes a multi-component signaling complex involving the STAT5B transcription factor and other signaling intermediates to mediate neuronal survival and neurite outgrowth. Understanding the mechanism of intercellular communication of GPCR signalling in the nervous system is crucial for normal brain development and regulation of adult neural processes. Thus, defining the molecular determinants that control opioid receptor signaling is important to address problems related to phenomena such as pain perception, tolerance and dependence that occur upon chronic opiate administration and define whether disruption of such interactions may contribute to the pathophysiology of nervous system related disorders and to the development of novel therapeutic strategies. This work was supported by the EU «*NORMOLIFE*» (LSHC-CT2006-037733) and the GSRT, Excellence II, grant 3722, "*NO-ALGOS*".

L302

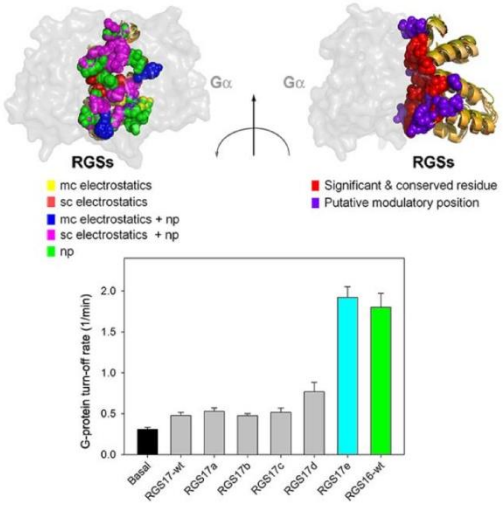
DECIPHERING AND RE-DESIGNING INTERACTION SPECIFICITY IN G-PROTEIN SIGNALING

Ali Asli[1], Mickey Kosloff[1]

1. *Department of Human Biology, Faculty of Natural Sciences, University of Haifa, Haifa, Israel.*

For signaling cascades to function correctly, their components must recognize their appropriate partners accurately. This requirement presents a challenge for living cells, as *related* components are used repeatedly in both parallel and intersecting cascades within the same cell. Signaling therefore requires that the interactions of *particular* protein-family members be tailored to each signaling cascade via interaction specificity, and this requirements is especially relevant in G-protein coupled signaling cascades. Understanding the structural basis for such selectivity is a major goal in both experimental and computational biology. Yet, beyond single representative examples, little is known of how specificity is determined among members of these large signaling protein families.

We developed a "bottom-up" approach to decipher interaction specificity – combining an experimental activity benchmark with a structure-based computational mapping of function/specificity determinants at the protein family level [1]. We applied this approach to heterotrimeric G-proteins and their interacting protein partners, with particular emphasis to G-protein–RGS interactions. The resulting residue-level maps are then used to redesign proteins with altered activities and specificities, offering new structural insights into G-protein signaling and paving the way for the rewiring of signaling networks at the cellular level.



References:

1.) Kosloff M. *et al.*, Nature Structure Molecular Biology, **2011**, 18 (7), 846-853.

L303

FORMATION OF THE ARRESTIN-GPCR COMPLEX: A TALE OF TWO LOOPS

Florent Beyrière, Martin Heck, Klaus Peter Hofmann, Ciara C.M. Lally, Patrick Scheerer, Martha E. Sommer, Michal Szczepek

Institute für Medizinische Physik und Biophysik (CC2), Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany

In G protein-coupled receptor (GPCR) signalling, arrestin is the "stop protein". Following phosphorylation of the active receptor, arrestin binds and thereby blocks further receptor coupling to G proteins. Arrestin binding to the active, phosphorylated receptor (R*P) is a multi-step process. Arrestin first interacts with the phosphorylated receptor C-terminus in a low affinity pre-complex, which primes arrestin for the subsequent transition to the high-affinity-complex with R*P. High resolution crystal structures are available for many different GPCRs in inactive and active states. Two recent crystal structures of pre-activated arrestin indicate what arrestin might look like in the pre-complex [1, 2]. Despite this wealth of structural information, we still do not understand the basic organization of the complex, nor how arrestin transitions from the pre-complex to the high-affinity complex.

In order to address these questions, we have applied multiple biophysical methods to arrestin-1 and rhodopsin, a GPCR of the visual system. We developed a model describing how arrestin employs distinct binding modes to interact with different functional forms of rhodopsin [3]. We identified two loops on arrestin, the finger loop in the N-domain and loop-344 in the C-domain, which mediate the binding modes by their different binding preferences.

More recently, we have gained insight into the molecular mechanisms governing the versatility of arrestin binding. A crystal structure of a peptide analogue of arrestin finger loop bound within the cytoplasmic crevice of the active receptor indicates how arrestin recognizes and stabilizes R* [4]. Site-directed fluorescence studies employing labelled arrestin mutants and quenching agents placed directly in the membrane suggest that loop-344 loop is a membrane anchor in both the pre-complex and the high-affinity complex. Notably, this loop shifts its orientation in the membrane during the transition from pre-complex to high affinity complex. We hypothesize that the simultaneous engagement of the receptor C-tail and the membrane by arrestin properly orients the pre-complex so that the finger loop can bind within the cytoplasmic crevice of R*P.

References:

1. Kim YJ, et al., *Crystal structure of pre-activated arrestin p44.* Nature, **2013**, 497, 142-6.
2. Shukla AK, et al., *Structure of active beta-arrestin-1 bound to a G-protein-coupled receptor phosphopeptide.* Nature, **2013**, 497, 137-41.
3. Sommer ME, Hofmann KP and Heck M, *Distinct loops in arrestin differentially regulate ligand binding within the GPCR opsin.* Nat Commun, **2012**, 3, 995.
4. Szczepek M, et al., *Crystal structure of a common GPCR binding interface for G protein and arrestin.* Nat Commun, **2014**, in press.

L304

FORMATION, PURIFICATION AND CHARACTERIZATION OF A PHARMACOLOGICALLY RELEVANT AND FUNCTIONALLY COMPETENT β2 ADRENERGIC RECEPTOR-βARRESTIN COMPLEX

Rosana I. Reis[1], Arun K. Shukla[2], Gerwin Westfield[3], Li-Yin Huang[2], Prachi Tripathi-Shukla[2], Seungkirl Ahn[2], Anthony A. Kossiakoff[4], Georgios Skiniotis[3], Robert J. Lefkowitz[2,5]

[1]*Department of Biochemistry, University of Oxford, Oxford, UK.*
[2]*Department of Medicine, Duke University Medical Center, Durham, North Carolina, USA.*
[3]*Life Sciences Institute and Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, USA.*
[4]*Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois, USA.*
[5]*Howard Hughes Medical Institute. Duke University Medical Center, Durham, North Carolina, USA.*

G Protein Coupled Receptors (GPCRs) are the main conduit of information transfer between cells and participate in virtually every physiological process through innumerous signaling pathways. GPCRs are critically regulated by β-arrestins (βarrs), which not only desensitize G protein signaling but also initiate additional signaling cascades on their own. Crystallography reveals exciting new details of GPCRs activation, including the β2 adrenergic receptor (β2AR)-G protein complex which has provided novel insights into the structural basis of receptor activation of G Proteins. However, a complementary set of structural studies on signaling through βarrs is still lacking, primarily due to challenges in obtaining stable receptor-βarr complexes for structural studies. Here, we present a novel strategy utilizing a conformationally selective antibody fragment to purify a biochemically stable, pharmacologically functional and physiologically relevant β2AR-βarr1 complex assembled in cells through co-expression of the receptor and βarr 1. This approach results in a complex which directly follows the ligand efficacy profile on β2AR. The amount of □arr co-purifying with the receptor also depends on the concentration of ligand and therefore correlates with the ligand occupancy. Analysis of this purified complex by single particle Electron Microscopy (EM) reveals excellent monodispersity and an optimal sample for subsequent structural characterization. The results described here should facilitate a wide range of structural studies on β2AR-βarr interaction which should ultimately provide the missing links to fully understand the structural basis of GPCR activation and regulation by βarrs.

L305

COMPUTATIONAL STUDY ON GPCR – G PROTEIN INTERFACES

Stefan Mordalski, Andrzej J. Bojarski

Institute of Pharmacology Polish Academy of Sciences, 12 Smetna St, Krakow, Poland

Recent studies show growing interest in targeting the cytoplasmatic interface of GPCRs with drugs [1,2]. New compounds, both petides and small molecules, prove promising pharmacological effects, however the detailed mechanism of interactions with intracellular interface of GPCRs has not been revealed yet.

Since the publication of the β2 Adrenergic receptor in complex with G_s protein is available [3], the aim of this study is to provide an information about other types of GPCRs interacting with G_i (Muscarinic M2 receptor and 5-HT_{1B} receptor) and G_o (5-HT_{2B} receptor) through bioinformatic analysis and molecular dynamics simulations.

Acknowledgements:

The study was partially supported by the grant PLATFORMEX (Pol-Nor/198887/73/2013) financed within the Polish-Norwegian Research Programme.

References:

1.) Dowal, L. et al., PNAS, **2011**, *108(7)*, 2951-6.
2.) O'Callaghan, K., Kuliopulos, A., Covic, L., J. Biol. Chem., **2012**, *287(16)*, 12787-96.
3.) Rasmussen, S. G., et al., Nature., **2011**, *477*, 549-555.

L306

STRUCTURAL STUDIES OF GPCRS

Chayne Piscitelli[1,2], Xavier Deupi[1,3], Gebhard F.X. Schertler[1,2]

1. *Laboratory of Biomolecular Research, Paul Scherrer Institute, 5232 Villigen-PSI, Switzerland*
2. *Department of Biology, ETH Zürich, 8093 Zürich, Switzerland*
3. *Condensed Matter Theory Group, Paul Scherrer Institute, 5232 Villigen-PSI, Switzerland*

Decades of GPCR research have yielded considerable insights into the cellular and molecular mechanisms of transmembrane signaling and associated physiological response pathways. Their central role in cellular physiology and pathology has led to GPCRs being one of the most prominent receptor families targeted for drug discovery efforts resulting in therapeutics for the treatment of many common diseases. Recently, discoveries in biased signaling mechanisms and advancements toward high-resolution structure determination of GPCRs have significantly updated the paradigm of GPCR related biology. In light of this, our lab is working with model GPCR systems to understand the structural mechanics of receptor activation, biased agonism, and ligand selectivity using a combination of X-ray crystallography, NMR, MD simulations, and other biophysical methods. In this talk, I will highlight some of our recent progress towards structural studies of peptide and adrenergic receptors, and will discuss the nascent results from studies probing the nature of ligand selectivity between beta-1 and beta-2 adrenergic receptors.

L307

DYNAMIC GPCR DIMERISATION SHOWN BY ENSEMBLE AND SINGLE MOLECULE FRET, AND DEER

_Patricia M. Dijkman[1], Oliver K. Castell[2,3], Alan D. Goddard[4], Mark I. Wallace[3], Anthony Watts[1]

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2. *Department of Chemistry, University of Oxford, 12 Mansfield Road, Oxford, OX1 3TA, United Kingdom*
3. *College of Biomedical & Life Sciences, Cardiff University, Heath Park, Cardiff, CF14 4XY, United Kingdom*
4. *School of Life Sciences, University of Lincoln, Brayford Pool, Lincoln, LN6 7TS, United Kingdom*

G protein-coupled receptors (GPCRs) are the largest class of eukaryotic membrane proteins. They are involved in many intracellular signaling cascades and are thus of great pharmaceutical interest, with ~40% of marketed drugs targeting GPCRs. It has been shown that GPCRs can form oligomers both *in vivo* and *in vitro*, affecting ligand binding and G protein coupling[1].

Neurotensin receptor 1 (NTS1) is one of few GPCRs that can be produced in *E. coli* in an active state, and has been implicated in conditions such as schizophrenia and Parkinson's and postulated as a biomarker for various cancers. NTS1 has been shown to dimerise in lipid bilayers[2], and though crystal structures of NTS1 in detergent have been recently published[3,4], there is still no structural data on the receptor and its dimer in a membrane environment.

By measuring intradimer distances between fluorescence or spin probes attached to engineered cysteines on NTS1, we aim to produce a model of its dimeric structure in a more native lipid environment. Using ensemble Förster resonance energy transfer (FRET) and double electron-electron resonance (DEER, also known as PELDOR) on NTS1 reconstituted into liposomes, in combination with single molecule FRET studies on NTS1 reconstituted into lipid droplet interface bilayers[5], we show that receptor dimerisation and the dimerisation interface are dynamic and transient.

References:

1.) Smith, N.J., Milligan G., Pharmacol Rev, **2010**, *62*
2.) Harding et al., Biophys J, **2009**, *96*
3.) White et al., Nature, **2012**, *490*
4.) Egloff et al., PNAS, **2014**, *111*
5.) Leptihn et al., Nat Protoc, **2013**, *8*

L201

MAPPING THE STRUCTURAL DETERMINANTS OF HISTAMINE RECEPTOR BINDING KINETICS

Rob Leurs, Albert J. Kooistra[1], Reggie Bosma[1], Sebastiaan Kuhne[1], Maikel Wijtmans[1], Tatsuro Shimamura[2,3], So Iwata[2,3], Rebecca C. Wade[4], Henry F. Vischer[1], Chris de Graaf[1], Iwan J.P. de Esch[1]

1. *Division of Medicinal Chemistry, Faculty of Sciences, Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), VU University Amsterdam, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands*
2. *Department of Cell Biology, Graduate School of Medicine, Kyoto University, Yoshidakonoe-cho, Sakyo-Ku, Kyoto 606-8501, Japan*
3. *Division of Molecular Biosciences, Membrane Protein Crystallography Group, Imperial College, London SW7 2AZ, U.K.*
4. *Molecular and Cellular Modeling Group, Heidelberg Institute for Theoretical Studies, Heidelberg, Schloss-Wolfsbrunnenweg 35, D-69118 Heidelberg, Germany*

This presentation will show how the recent breakthroughs in GPCR structural biology can be complemented by computational and experimental medicinal chemistry studies for a more accurate description and prediction of molecular and structural determinants of ligand binding kinetics in different key binding regions of the histamine receptors. Biocomputational approaches including structure-based virtual screening, protein-ligand interaction fingerprint scoring and customized molecular dynamics simulation techniques are used to discover and guide the design and synthesis of new GPCR ligands and elucidate the molecular mechanism of histamine receptor ligand binding kinetics.

L202

Characterization of internalization of the human GIP receptor enables discovery of a biased agonist

Sadek Ismail[1], Ingrid Dubois-Vedrenne[1], Marie Laval[1], Romina D'Angelo[2], Claire Sanchez[1], Pascal Clerc[1], Marie-Julie Gherardi[1], Véronique Gigoux[1], Remi Magnan[1] and Daniel Fourmy[1]

[1] Université de Toulouse 3, EA 4552, INSERM U1048/I2MC, Toulouse, France.
[2] Cellular Imaging Facility Rangueil (I2MC/TRI Plateform).

Incretin receptors are essential for regulation of glucose homeostasis through binding to specific G-protein-coupled receptors making them molecular targets for clinical applications. Thus, efforts directed at understanding the cellular and molecular mechanism by which natural hormones and pharmacological agents regulate their presence at the cell surface and their activity is of paramount importance.

Herein, we have investigated signalling, internalization and intracellular trafficking of the human Glucose-Insulinotropic Polypeptide receptor (GIPR). GIP, a full agonist on GIP-induced production of cyclic AMP, stimulated rapid robust internalization. Only a minority of internalized GIPR recycled to cell surface, the major part being directed to lysosomal degradation pathway. GIPR internalization mostly involved clathrin-coated pits, AP-2 and dynamin. However, unlike many G-protein-coupled receptors, neither C-terminal region of the GIPR that contains phosphorylation sites, nor β-arrestin1/2 was required for internalization. GIP-induced cAMP production originated mostly from plasma membrane-embeded GIPR. However, a minor part of cAMP was generated from GIPR located in early endosomes. Moreover, a biased agonist of the GIPR, N-acetyl-GIP, which fully stimulated cAMP production and insulin secretion from MIN-6-B1 cells but did not stimulate internalization of the GIPR, was identified. The rate of GIPR-mediated signaling desensitization by N-acetyl-GIP was very low compared to that by GIP. Since β-arrestins do not participate in rapid internalization of GIP-induced GIPR, the biased agonist would differently engage Gs and possibly clathrin adaptor, or some other proteins recruiting active GIPR to clathrin-coated pits. All these data should be useful for a better understanding of pathophysiology of diabetes type II and for design of new incretin molecules.

L203

BIASED SIGNALING AT CHEMOKINE RECEPTORS DOES NOT FOLLOW THE G-PROTEIN VERSUS β-ARRESTIN PARADIGM

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Biased signaling is a novel and promising concept, which challenges the present understanding of GPCRs function and raises new opportunities for the use of these receptors as therapeutic targets [1]. Therefore, it appears important to identify ligands and receptors that exhibit biased signaling properties, and to understand how biased agonist binding induces the selective activation of a signaling pathway. Although the present dogma states that biased signaling relies mainly on the selective activation of G protein- or arrestin-dependent pathways, our data indicate that signaling bias is much more complex than initially thought. We investigated the coupling selectivity of chemokine receptors CCR2, CCR5 and CCR7 in response to various ligands with G protein subtypes by using BRET biosensors monitoring directly the activation of G proteins. We also compared data obtained with G-protein biosensors to those obtained with other functional readouts, such as β-arrestin-2 recruitment, cAMP accumulation and calcium mobilization assays. We showed that the binding of chemokines to CCR2, CCR5 and CCR7 activated the three Gαi subtypes (Gαi1, Gαi2 and Gαi3) and the two Gαo isoforms (Gαoa and Gαob) with potencies that generally correlate to their binding affinities. In addition, we showed that the binding of chemokines to CCR5 and CCR2 also activated Gα12, but not Gα13. For each receptor, we showed that the relative potency of agonist chemokines was not identical in all assays, supporting the notion that signaling bias exists at chemokine receptors. However, these signaling biases were sometimes subtle and did not rely entirely on the relative ability of the ligand-receptor complex to recruit either G proteins or β-arrestins. This is particularly true for CCR7 activation by CCL19 and CCL21, which was until now considered to be a prototypical example of signaling bias amongst natural ligands [2-4].

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L204

PROBING A MOLECULAR DETERMINANT FOR BIASED AGONISM AT THE β_2-ADRENOCEPTOR

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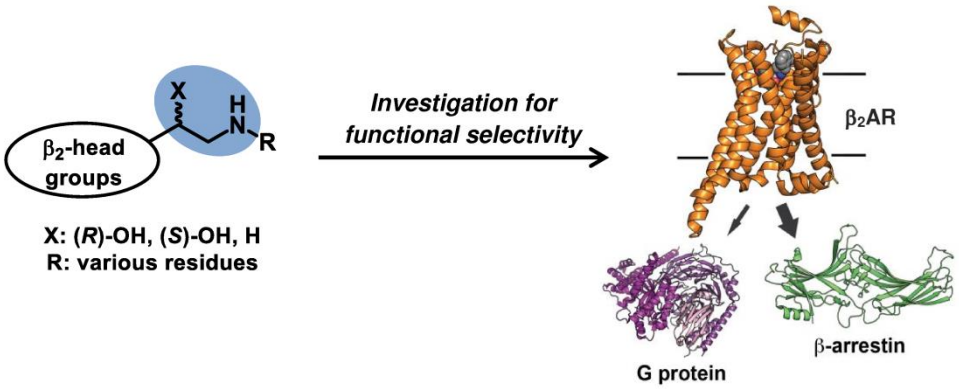
The differential activation of specific signaling pathways by G protein-coupled receptors (GPCRs) has been extensively studied and it is now commonly accepted that ligands may induce distinct receptor conformations, thus mediating preferential coupling to intracellular effectors proteins.[1,2] For the β_2-adrenoceptor (β_2AR), several clinically used drugs had been identified to be functional selective ligands.[3] Most strikingly, it was found that stereoisomers of fenoterol, a β_2-agonist that is used in the treatment of asthma, promote differential coupling to two Gα subunits.[4] These isomers only differ in the configuration of the β-hydroxyl group, which is part of the amino alcohol moiety, a crucial recognition element of β_2-agonists. Because the molecular basis of such ligand-selective signaling is still poorly understood, we planned to further evaluate the potential of distinct stereoisomers of β_2AR ligands to differentially activate specific signaling pathways. We therefore designed and synthesized a series of ligands based on β_2-(inverse)agonist and antagonist scaffolds that feature both configurations of the β-hydroxyl group, as well as the ligands devoid of this moiety and tested them for their ability promote G protein or β-arrestin coupling. The experiments indicated stereospecific recognition of the ligands with similar maximal effects of both enantiomers at the β_2AR.



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LP02

NANOBODY-ENABLED FRAGMENT SCREENING ON ACTIVE-STATE CONSTRAINED GPCRS

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In the past 10 years, GPCR drug discovery has relied on cell-based assays combined with high-throughput screening (HTS) of large compound libraries for lead discovery as well as optimization. However, progress in identifying new small molecule drugs has been disappointing and the pace of GPCR drug discovery is slow. One key problem is that compounds do not only need to target the correct GPCR, but the drugs must also exhibit the appropriate efficacy profile: agonist, partial agonist, neutral antagonist or inverse agonist. Even worse, hits from HTS screens frequently must be deconstructed to remove liabilities that cause toxicity or non-ideal ADME properties.

Fragment-based drug discovery uses low-molecular-weight, moderately lipophilic, and highly soluble fragments as starting points for developing novel drugs. FBDD is particularly advantageous for its ability to more completely assess “compound space” for molecules that interact with the target of interest. Last years, our lab has shown that Nanobodies are effective tools for stabilizing agonist-bound active states of GPCRs. Building on this technology, we have developed a Nanobody-enabled fragment screening approach to explore new chemical space for the development of drugs targeting GPCRs. Our approach has the competitive advantage to other methods that we can screen fragments that exclusively bind to particular functional conformations of the receptor allowing us to triage our fragments according to efficacy profile and potency from a single biophysical assay. Nanobody-enabled screening of a moderate sized fragment library of 1000 compounds led to the discovery of several fragments with an agonist efficacy profile.

L401

NOVEL AGONISTS FOR THE DOPAMINE D_3 RECEPTOR SUBTYPE WITH HIGH IN VIVO ACTIVITY

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L-DOPA is still the gold standard treatment for motor functions with dopamine substitution therapy in patients with Parkinson´s disease. Dopamine receptor subtype agonists have great influence on therapeutic options as an ideal dopamine receptor agonist should fulfill the following criteria: 1) a physiological receptor profile with good anti-parkinson efficacy, 2) a good brain distribution, 3) oral bioavailability, 4) rapid onset, 5) long acting and 6) no unwanted side-effects. Although a small number of non-ergot derivatives are on the market, no single drug available fulfills all the criteria.

In a long-termed development program we have changed the 2-aminothiazole motif of pramipexole as a prototypical catechol bioisosteric moiety by removing the aromatic amino functionality as described previously with etrabamine [1,2]. This derivatisation maintained or improved affinity at dopamine D_3 receptor subtype, maintained agonist properties and simulated binding profile at dopamine D_2-like receptor family. Depending on the substitution pattern on the core pharmacophore element a series of highly affine and selective agonists have been developed. Selected compounds were screened on unilateral 6-OHDA-lesioned rat model of Parkinsons´s disease and further selection on MTPP-treated marmoset model for their antiparkinsonian efficacy in comparison to L-DOPA, apomorphine and ropinerole. At least two compounds simultaneously fulfilled all the criteria mentioned above and showed high drug potential due to the results of the initial preclinical toxicological screenings. Studies on functional signaling based on [^{35}S]GTPγS shift and on $ERK_{1/2}$ phosphorylation showed significant differences and a good predictive factor for in vivo activities.

Support by the Alexander-von-Humboldt foundation, the EU COST Actions CM1103 and 1207 as well as the DFG INST 208/664-1 FUGG is greatly acknowledged.

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L402

FLUORESCENT PROBES TO TRACK GPCR BINDING AND DIMERIZATION.

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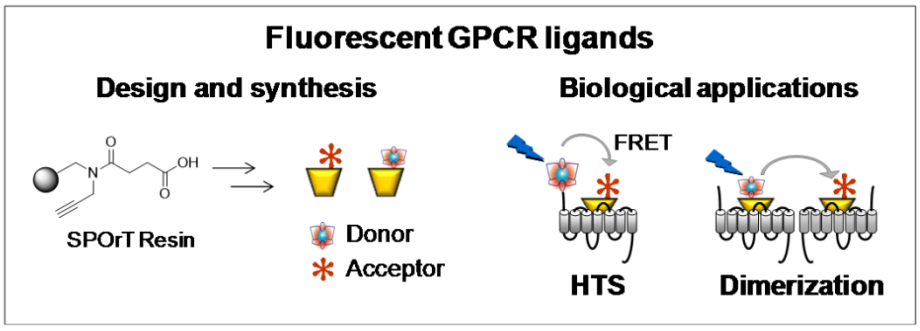
G-protein-coupled receptors (GPCR) represent the largest family of cell surface membrane proteins encoded by the human genome and more than 40% of all marketed therapeutics act on them. However, these drugs target only few members of the family (15%). So, there is an enormous potential to exploit the remaining family members, including the orphan receptors for which no existing ligands have so far been identified. Besides, in the last decade, homo- and hetero-oligomerization of GPCRs have been described as a new way to modulate receptor pharmacology and functional activity. Thus, heteromer-targetted drug discovery opens new perspectives both in Academic pursuits and for the Pharmaceutical industry.

In this context, we have set up innovative fluorescent-based assays in order to gain a better understanding of GPCR functional architecture but also to set up new receptor-selective high-throughput screening (HTS) assays for classical, orphan and heterodimeric GPCRs. Owing to their high sensitivity and to their reduced environmental safety risk, fluorescent technologies represent a powerful molecular tool to study ligand-GPCR interactions (1). However, the prerequisite to develop such methods is to design and to synthesize high affinity and selective fluorescent probes.

As we will illustrate, synthetic methods have been set up to facilitate the access to original fluorescent GPCR probes with potential applications in drug discovery. For instance, the first environment sensitive ("Turn-on") probe was developed to detect and to monitor oxytocin GPCR at the surface of living cells (2). We have also designed and synthesized fluorescent compound-based libraries allowing the discovery by FRET of the first non-peptidic agonist of the apelin receptor (3). Finally, selective fluorescent ligands were developed to detect vasopressin V_{1a}-V_2 heterodimers at the cell surface and to set up a novel TR-FRET assay to screen for heterodimers (4).



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L403

ANNELATED XANTHINES AS A1/A2A/A2B ADENOSINE RECEPTORS LIGANDS

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To date four adenosine receptor (AR) subtypes designated A_1, A_{2A}, A_{2B} and A_3 have been cloned and pharmacologically characterized. A_1 and A_{2A} ligands are currently being developed as promising therapeutic agents for CNS disorders (especially A_{2A} antagonists - utility in Parkinson's disease and cancer therapy), cardiac failure, depression and addictions. Use of adenosine A_{2B} antagonists in the therapy of pain, asthma or related pulmonary diseases and diabetes has been proposed[1,2].

Our studies are focused on the search for ligands of adenosine receptors A_1, A_{2A}, A_{2B} in the group of tricyclic derivatives of xanthines of the type of: imidazo-, pyrimido- and diazepinopurine-2,6-diones[3,4]. Compounds with (non)selective activity at these three types of receptors have been found using *in vitro* tests on as well rat as human receptors expressed in CHO cells (as radioligands [^{3}H]CCPA, [^{3}H]MSX-2 and [^{3}H]PSB-603 were used for A_1, A_{2A} and A_{2B} receptors respectively). Several compounds were *in silico* (physicochemical properties (clogP, logS, tPSA) toxic effects (service OSIRIS) and blood-brain barrier penetration (models of Zhao, Norinder-Haeberlein or Lipinski),and experimentally examined for their druglikness in the tests evaluating their cytotoxic properties and influence on CYP3A4 cytochrome activity. To study their potential cytotoxicity, antiproliferative effects of selected compounds on HEK-293 cells was tested.

Molecular modelling and docking methods have been applied to predict possible modes of the obtained derivatives binding inside the pocket of the human A_{2A} and A_{2B} receptors. physicochemical properties (clogP, logS, tPSA), toxic effects (service OSIRIS) and blood-brain barrier penetration (models of Zhao, Norinder-Haeberlein or Lipinski) [2]. To study their potential cytotoxicity, antiproliferative effects of selected compounds on HEK-293 cells was tested. Additionally, to predict potential drug-drug interactions, effects on cytochrome CYP3A4 enzyme activity was examined.

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L404

NOVEL RELEASE OF THE GPCR-SSFE PLATTFORM FOR AUTOMATIC MULTIPLE-TEMPLATE BASED GPCR HOMOLOGY MODELING

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The initial version of the GPCR Sequence-Structure-Feature-Extractor (GPCR-SSFE), a webserver for multiple-template based homology model generation, was published in 2011 (accessible at http://www.ssfa-7tmr.de/ssfe, [1]). The recent major update integrates representative crystal structures for all distinct rhodopsin-like GPCRs in the inactive state, which are available as of yet. Additionally, the dataset of precalculated GPCR homology models was expanded to enable comprehensive statistical analyses. Novel ideas for database optimization and enhancement were developed, e.g. the implementation of a new template selection strategy which is based on a fingerprint correlation scoring approach. In comparison to other approaches, the uniqueness of this method is that the template selection is carried out for each transmembrane helix rather than for the receptor as a whole by finally assembling all helices together. This separate helix assembly for generating multi-template GPCR homology models was retained, as recent studies have confirmed that using multiple structures instead of a single one leads to more accurate results. Due to the selection of the most appropriate template per helix, one covers the structural diversity of the entire set of available crystal structures. Thereby, specific sequence and structural features can be incorporated in the selection process to increase the feature and alignment coverage of the given GPCR.

Apart from the opportunity to model your protein of interest the GPCR-SSFE 2.0 resource stores 20500 GPCR sequences from various species. This huge sequence dataset, together with the pre-calculation of homology models for the same number of GPCRs, will facilitate statistical analyses of variant and conserved positions at every helix position for a certain GPCR. This in turn facilitates the identification of functional residues for processes such as ligand binding, G protein- or β-arrestin interaction.

Recently published crystal structures provided us with an ideal means of assessing the performance of GPCR-SSFE 2.0. Indeed, the models produced by our updated approach have clearly improved in comparison to the 2011 version. Another proof of principle for applying this multiple-template based approach supports experimental data, which have shown differences in ligand binding of ETAR and ETBR. Previously, single template approaches had failed to predict these differences, since the same template was chosen to model both receptors due to their high sequence similarity. The divergent ligand binding behaviour can only be structurally explained by the appropriate template selection for each helix separately combining multiple templates.

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L405

UGI-BASED ENGINEERING PROVIDES CONVERGENT ASSEMBLY AND NOVEL ARCHITECTURES FOR HOMOBIVALENT LIGANDS

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A plethora of biochemical, structural and biophysical evidences are progressively confirming GPCR´s propensity to intermolecular associability [1]. GPCR dimers/oligomers often display unique ligand binding profile, distinct phenotypic trafficking and altered signalling properties, which suggest they could be conceptually novel targets for the treatment of complex diseases [2]. With insights revealing novel GPCR dimers and high order oligomers continuously emerging; the comprehensive study of the molecular basis of its function and pathophysiological implications, but also the discovery of ligands enabling its fine modulation remain challenging endeavours. Herein we document a series of bivalent ligands targeting $5HT_{2A}$ receptors, derived of the 2,5-dimethoxy-4-iodoamphetamine (DOI), that exhibit unexplored architectures and are assembled through an Ugi four-component reaction. The Ugi-based engineering (Figure 1) provides a versatile and experimentally simple conjugation strategy, while introduces a novel paradigm in bivalent ligand design: the concept of tunable multi-center linkers.

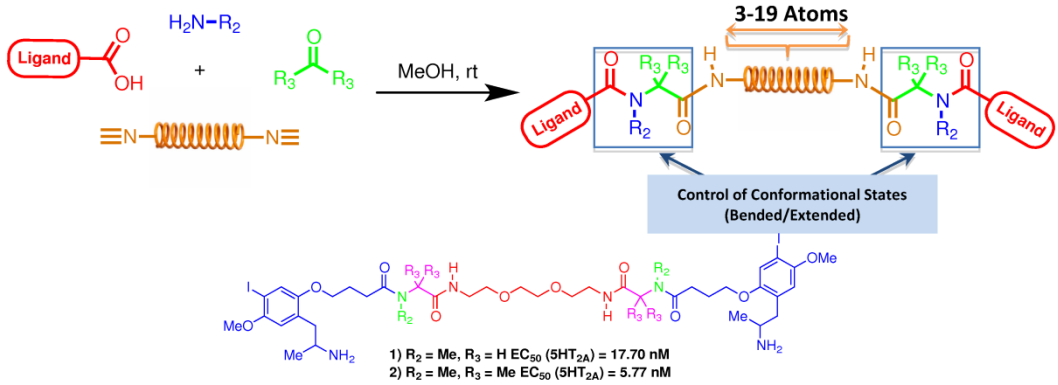

Figure 1. Synthetic strategy and structure of targeted homobivalent ligands.

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L406

IN-SILICO TARGETING OF OBESITY: THE CASE OF THE HUMAN BILE ACID RECEPTOR

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The human G protein-coupled receptor of bile acids has been proposed to be a molecular target for metabolic syndrome, diabetes and obesity. This receptor is ubiquitously expressed in the human body, but at higher levels in the gallbladder. It binds to endogenous bile acids, and its activation increases energy expenditure, lipid metabolism and glucose-tolerance, while at the same time it decreases serum glucose and insulin levels. There are no known marketed drugs that target the bile acid receptor and its crystal structure is not known. Here, we built and validated a computational model of the bile acid receptor, and used it for the *in-silico* screening of non-steroidal molecules. Initial experimental testing of the *in-silico* hits validated four non-steroidal compounds which were used to refine our computational model and select a new generation of compounds for experimental validation.

L407

EVOLUTIONARY HUBS IN SELECTED GPCR FAMILIES

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Class A G-protein-coupled receptors represent the widest transmembrane receptor family in the human genome. Its 300 non-olfactory members can be classified into a dozen of subfamilies. To gain insight into the mechanisms that drove GPCR evolution and gave each subfamily its specificity, we analyzed the sequence covariation of selected GPCR sequence sets. We previously showed [1] that, among different methods to analyze covariation between positions in a multiple sequence alignment, one method called OMES (Observed minus Expected Squared) [2] is especially suited to analyze covariation related to the emergence of sub-families within a protein family. This method favors coevolving pairs with intermediate conservation and a networking structure of coevolving residues with a central residue involved in several high scoring pairs. Applied to selected sets of GPCRs, we show that the central residue corresponds to a residue known to be crucial for the evolution of the GPCR family and the subfamily specificity. This central residue can be viewed as an evolutionary hub whose mutation was necessary but not sufficient for the divergence to occur. Knowledge of coevolving residues provides further information on the specificity of each subfamily and the mechanisms that made possible the diversification of the GPCR family.

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L408

GLISTEN DB : YOUR TURN TO PLAY!

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GLISTEN COST constitutes an interdisciplinary network of researchers who all study GPCRs yet using different approaches, focus or tools. Aiming at facilitating new collaborations between GLISTENers, the GLISTEN DB was designed as an inventory of skills, materials and expertises. In particular, it can host (1) structure and availability of chemical compounds, (2) description and cost of biological assays, and (3) eventually, if chemical compounds are indeed tested on biological assays, activity data points.

During GLISTEN meeting in Barcelona (Spring 2014), WG4 members have agreed on the structure and contents of the database: an open web platform collecting information on GPCR-based assays and on known or potentially bioactive compounds.

We have now created the database, which can be filled, modified or simply queried by users. In this presentation, you will learn how to access the database, how to contribute by adding/modifying data, and how to query the database.

L101

Tuning of Angiotensin II Selectivity to its GPCRs AT1R and AT2R

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G-protein-coupled receptor (GPCR) subtypes posses distinct functional and pharmacological profiles, thus development of subtype selective ligands has great therapeutic potential. This is especially the case for the angiotensin receptor subtypes AT1R and AT2R that exert functional opposing effects, and highlighted AT2R activation as an important cancer drug target. Here we describe a strategy to fine-tune ligand selectivity for the AT2R/AT1R subtypes through control of ligands aromatic-prolyl interactions. Through this strategy an AT2R high affinity (Ki=3 nM) agonist analogue which exerted 18.000-fold higher selectivity for the AT2R versus the AT1R was obtained. We show that this compound is able to inhibit MCF-7 breast carcinoma cellular proliferation in the low nanomolar range.

L102

PROTEIN BACKBONE NMR REVEALS LIGAND RECOGNITION, SIGNAL TRANSMISSION, AND QUENCHING OF MOTIONS BY THERMOSTABILIZATION IN THE β1-ADRENERGIC RECEPTOR

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G protein-coupled receptors (GPCRs) are membrane signal transmission devices that trigger intracellular responses to binding of extracellular ligands. Despite recent breakthroughs in GPCR crystallography mainly based on conformational stabilization by protein engineering, the details of ligand-induced signaling are not well understood due to missing dynamical information. In principle, such information could be provided by NMR, but so far the quality of NMR spectra obtained on eukaryotic GPCRs has been very low and only limited information on the dynamics of a few side chain sites with favorable spectroscopic properties could be obtained. Here, we show that receptor backbone motions can be followed at virtually any backbone site in a thermostabilized mutant of the □1-adrenergic receptor by the introduction of 15N-labeled amino acids in a eukaryotic expression system. Labeling by 15N-valine provides over twenty resolved resonances that report on structure and dynamics in six ligand complexes and the apo form. The response to ligands in the vicinity of the binding pocket shows ligand-specific deformations according to their chemical structure and affinity, whereas the response at the intracellular side of helix 5 correlates linearly with ligand efficacy for the G protein pathway. Presumably as a consequence of thermostabilization, response to ligands is weak in helix 6, which is expected to undergo large motions during receptor activation. An additional highly thermostabilizing mutation abrogates part of the response to the partial agonist cyanopindolol, giving specific evidence how thermostabilization restricts GPCR flexibility..

L103

CORRELATED MOTIONS IN DISTINCTIVE CONFORMATIONAL STATES OF THE CHEMOKINE RECEPTOR CXCR4

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Inactive and active structures are available for several members of the GPCR family. These structures together with dynamics studies have provided deeper insights into functional dynamics of GPCRs. However, intermediate conformations and transition pathways between the two states remain unclear.

For the human CXC chemokine receptor type 4 (CXCR4), only crystallographic structures of inactive homodimers are available. Consequently, the different conformational states of CXCR4, the correlated motions between these states and the activation mechanisms of this receptor remain to be elucidated. In the current study, we have performed (classical and accelerated) molecular dynamics simulations, utilising the crystal structure of CXCR4 in inactive state and homology model of CXCR4 in active state, in order to understand the structural elements of the activation mechanism of this receptor.

Comparison of movements in active and inactive CXCR4 conformations from molecular dynamics simulations has given insights into the distinctive conformational flexibility of these states. Particularly, correlated motions, characterising the dynamics of different conformational states of CXCR4, have been highlighted. In addition, the inactivation of the active CXCR4 model, sampled through accelerated molecular dynamics, highlighted the role of key residues involved in deactivation of the G-protein binding site. Finally, we highlighted time-dependent correlated conformational changes of side chains surrounding functional GPCR microswitch residues that trigger the transition from the active to the inactive state of the CXCR4 receptor.

L104

SINGLE-MOLECULE MICROSCOPY DECIPHERS THE RELATION BETWEEN TRAFFICKING AND SIGNALING OF THE NK1 RECEPTOR IN LIVING CELLS

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Lateral diffusion enables efficient interactions between membrane proteins leading to the transmission of chemical signals within and across the plasma membrane. An open question is how cell surface receptors are distributed over space and time and thereby influence the transmembrane signalling network. Here we study the mobility of a prototypical G protein-coupled receptor (GPCR), the neurokinin-1 receptor (NK1R), during its different phases of cellular signalling. Investigating thousands of single NK1R trajectories reveal a very characteristic and very broad mobility distribution pattern with two major receptor populations, one showing high mobility and low lateral restriction, the other low mobility and high restriction. The mobility distribution pattern changes in a characteristic manner after agonist binding leading to receptor (i) activation, (ii) desensitization, (iii) endocytosis, and (iv) recycling. The study uncovers the functional role of the heterogeneous spatiotemporal distribution of individual GPCRs during the entire cellular signalling cycle.

L105

NEW FEATURES FOR GPCR MODELING IMPLEMENTED ON GPCR-MODSIM AND PYMEMDYN

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The GPCR-Modsim webserver [1] and the derived standalone python package py**MEM**dyn [2] are services used worldwide for research and teaching. We herein present the current status and recent updates of the GPCR-Modsim project which has been recently migrated to Uppsala University, and it's now located at http://**gpcr-modsim.org**. py**MEM**dyn can be obtained freely upon request from bitbucket.org.

We present an update the available GPCR crystal structures used as supported templates. This includes a manually curated structural alignment, as well as a revised conformational clasification into inactive, partially active, and active structures. The standalone py**MEM**dyn python package for setting up GPCR membrane and solvent embeded molecular dynamics (MD) simulations has been updated for flawless integration into the family of gromacs 4.6.X programs, which is present in most computational cluster facilities across Europe. Compatibility with gromacs 5.0 is under development.

The pipeline for MD equilibration has been updated in order to consider the consensus transmembrane contacts in Class A receptors [3]. With this protocol, new modeled structures will be allowed to undergo biophysical relaxation, which is otherwise hardly influenced from the initial template structure used in the homology modeling process, while maintaining the general features typical of class-A GPCR topology. The interhelical contacts described by Venkatakrishnan and collaborators [3] are considered as smooth distance restraints, whose strength is proportional to the intensity and frequency of the given contact in the available GPCR structures. Ballesteros-Weinstein notation has been implemented to guarantee the unique identification of the positions involved for new modeled sequences (or a new PDB structure uploaded to the webserver). This protocol is a unique combination of Homology and first-principles modeling techniques with potential advantages for GPCR sequences which have only distant homologs available as templates.

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Poster abstracts

Numbering: Pxyy, P-poster, x- Working Group number or G for GPCRDB meeting, yy- sequential number

P101

FREE ENERGY SURFACE OF OXYTOCIN AND HOMOLOGY MODELING OF OXYTOCIN RECEPTOR

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Oxytocin is a 9 residues long, neurohypophysial hormone, with several known biological roles during maternal bonding, labours, sexual reproduction and others. However, binding to its receptor, which is member of GPCR family, and the detailed structure of the receptor are not well known. Here, well-tempered metadynamics, in combination with dimensionality reduction techniques, was used to explore conformational free energy surface of oxytocin. Simulations were performed in both implicit and explicit solvents. Three different energy minima were found and so we assume that this hormone has three stable conformers. Possible reason for different conformations of oxytocin could lie in specific structure of its receptor including existence of sequences from several loops making the ligand pocket. In order to possiblly explain this we are currently working on oxytocin receptor model using homology modeling.

P102

CONFORMATIONAL FREE ENERGY MODELLING OF SPECIALIZED PRO-RESOLVING MEDIATORS

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Specialized pro-resolving mediators (SPMs) are a new class of signaling molecules and GPCR ligands involved in resolution of the inflammation process. These lipid-derived molecules are highly flexible and can adopt many distinct conformations. Their conformational equilibria can be influenced by environment, i.e. solvent, membrane etc. Conformational equilibria of a molecule can be expressed in terms of a conformational free energy surface. It can be calculated by molecular dynamics simulation, however, this requires very long simulation times due to poor sampling provided by this method. Here we report application of an enhanced sampling method called metadynamics to predict conformational equilibria of five selected SPMs. The results are compared with long reference molecular dynamics simulations. The effect of environment on SPMs' conformations is also discussed.

P103

TOWARDS UNDERSTANDING GPCR-LIGAND BINDING: SELECTIVITY, FUNCTION, AND BINDING KINETICS

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This poster will show how the recent breakthroughs in GPCR structural biology can be complemented by computational and experimental studies for a more accurate description and prediction of molecular and structural determinants of ligand binding kinetics, functional activity, and selectivity in different key binding regions of GPCRs. Biocomputational approaches including structure-based virtual screening, protein-ligand interaction fingerprint scoring and customized molecular dynamics simulation techniques are used to discover new GPCR ligands and elucidate the molecular mechanism of GPCR ligand binding.

P104

A PREFERRED BINDING MODE EMERGES FROM THE MODELING OF THE OREXIN-A – OX_1R COMPLEX

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Orexin receptors OX_1 and OX_2 are activated by peptides orexin-A and -B.[1] The orexin system acts as a gatekeeper in the regulation of sleep and feeding patterns. There is also evidence of favorable orexin effects in certain cancer cells. No potent small molecule agonists are available, which would be critical for further biochemical and physiological characterization of the receptors. An agonist might also be a useful drug against narcolepsy and possibly some cancers. Few antagonists have reached clinical trials against insomnia, but none has reached market. An antagonist could also be useful against obesity. Interactions between the orexin peptides and receptors are not known, which has hampered the search for small molecular ligands.

Binding interactions of the orexin peptides have mainly been studied by point mutations to the peptides or to the receptors. Computational modeling has not been applied to the orexin system, and distinct binding interactions for the peptides, let alone complete binding modes, have not been previously suggested. In order to study these interactions, we have conducted an exhaustive docking of orexin-A_{15-33} with ZDOCK [2] and RDOCK [3] to three homology models of orexin receptor 1. Models were derived from neurotensin receptor 1 [4], chemokine receptor 4 [5] and a hybrid structure of the two. The best ranked docking poses show a consistent binding mode which is well in line with previous mutation studies. The binding mode we propose can be used to design new mutation studies, and it will contribute to the understanding of orexin receptor activation.

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P301

CHARACTERIZATION OF THE HOMODIMERIZATION INTERFACE OF THE ADENOSINE A_3 RECEPTOR

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In recent years, G protein-coupled receptors (GPCR) have been proposed to function as multiple units, ranging from dimers (homo and hetero) as well as oligomers. Dimerization may alter receptor pharmacology, signaling, trafficking, subcellular localization, and desensibilization [1,2]. In this context, we explore different homodimer models of A_3 Adenosine receptors by protein-protein docking and all-atom molecular dynamics simulations in the physiological environment of a lipid bilayer. Finally, this work analyses the structural and dynamic role of the key residues involved in the interface contact of the homodimer, as well as the major protein motions. This project will aid future structure-based drug design efforts on this pharmacological target.

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P302

COMBINED ANALYSIS OF β_2-ADRENERGIC /INSULIN RECEPTOR HETERODIMER BY RECEPTOR-HETEROMER INVESTIGATION TECHNOLOGY (HIT) AND BIOINFORMATICS

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Glucose metabolism is under cooperative regulation of both insulin receptor (IR) and β2-adrenergic receptor (β_2-AR) representing the receptor tyrosine kinases (RTKs) and the seven transmembrane receptors (7TMRs), respectively. The cross-talk between these different classes of receptors may occur at the 7TMR/RTK level or via various intracellular effectors/adaptor molecules including β-arrestin 1 and 2 (β –arr1 and 2). Evidence for the IR/β_2-AR heterodimerization was first provided by the by the receptor-heteromer investigation technology (HIT) showing isoproterenol- but not insulin-induced GFP^2-β-arr2 recruitment to the heteromer complex consisting of IR-Rluc8 and untagged β_2-AR; IR/β-arr2 interaction was only found to be constitutive. To investigate the specificity of IR and βarr2 interaction we used GFP^2-βarr2 1-185 mutant that lacks previously proposed binding region (amino acids 186-409) for the interaction with the IR. The BRET signal obtained in cells coexpressing IR-RLuc8 and GFP^2-βarr2 1-185 was significantly smaller (8.24 ± 0.71 mBU) compared to constitutive IR-RLuc8: GFP^2-βarr2 BRET signal (22.96± 2.18 mBU). Binding of the GFP2-βarr2 1-185 mutant to the β2AR was also significantly reduced.

To complement BRET data we next applied informational spectrum method (ISM), a virtual spectroscopy method for investigation of protein-protein interactions. *In silico* analysis confirmed β-arr2 involment in the heteromer complex and found that the affinity for complex occurence is higher for β_2AR:β_2AR:IR:β-arr2 than for β_2AR:IR:IR:β-arr2, which supports our previous findings of higher propensity of β_2-AR for heteromerization in higher order oligomers. In addition bioinformatics analysis showed that the affinity for interaction is lower among βarr2 1-185 mutant and β_2AR and IR compared to WT βarr2. In the complex (β2AR β2AR IR β-arr2), β-arr2 has higher affinity for binding to β_2AR.

In summary HIT and bioinformatics analysis provide evidence for i) IR/β2-AR heterodimerization, ii) specificity of constitutive IR:βarr2 interaction and iii) higher affinity of the β2AR for β-arr2.

P303

THEORETICAL STUDY OF PROTEIN-PROTEIN INTERACTIONS FOR OLIGOMERIC GPCRs

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The G-Protein Coupled Receptors (GPCRs), also called the seven-transmembrane receptors (7TM), are membrane proteins that create homo- and hetero-oligomeric systems. GPCRs play an important role in a signal transduction from extracellular side of a cell to its interior and possibly a formation of oligomers can modulate these transduction processes.

The molecular dynamics (MD) simulations [1] for systems in all-atom representation allow to study such systems in nano scale (both in space and time), but a large number of atoms in oligomeric systems precludes longer MD simulations. Currently, it is limited to a millisecond time scale for monomeric membrane proteins. This drawback can be overcome with more and more powerful supercomputers, however, studying dynamics of larger biological ensamblies are still beyond the scope of all-atom MD simulations. To address this problem, a development of novel coarse-grained methods [2] of MD, where several heavy atoms are being mapped onto one bead, is necessary, especially for membrane proteins. The simplified methods may help to investigate such protein complexes in long time scales. The developed coarse-grained method was additionally based on implicit membrane model (IMM [3]) representing solvent as a continuous medium. This method was used to investigate large oligomers of bacteriorhodopsin as well as to test stability of different GPCR dimers.

We also studied a formation of GPCR oligomers in monoolein cubic phase to get knowledge of formation of GPCR crystals in this atypical environment, since lipid cubic phase of monoolein are commonly used for crystallization of many membrane protein, and especially GPCRs.

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P304

INTERACTIONS DURING THE EARLY EVENTS OF GPCR SIGNALLING

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The G protein-coupled receptor (GPCR), neurotensin receptor type 1 (NTS1), is pharmacologically important and activated by the tridecapeptide hormone, neurotensin (NT), initiating a cascade of interactions through G proteins to effect cellular responses. The mechanisms by which these occur have only recently begun to be examined structurally, and standard assays involving downstream effectors or radioactive GTPγS G protein activation to describe GPCR-G protein interactions do not assay the interactions directly. Two methods have been used here to study the interaction of NTS1 with the signalling partners, $G\alpha_s$ and $G\alpha_{i1}$. A novel DNA-nanotechnological approach for preparing samples for electron microscopy (EM) has been used to study NTS1 and $G\alpha_{i1}$, both separately and complexed, on a functionalised 2D DNA lattice, providing the first direct evidence of this interaction [1].

A further nanotechnological approach, using the increasingly popular method of reconstitution of membrane proteins in nanodiscs, has been used to study NTS1-$G\alpha_s$ and NTS1-$G\alpha_{i1}$ interactions, using EM and surface plasmon resonance (SPR). The ligand (NT) affinity of detergent-solubilised NTS1 and NTS1 reconstituted into nanodiscs was ~1 nM, and for the first time, the affinity of binding of $G\alpha_{i1}$ and $G\alpha_s$ to NTS1 was directly measured and determined as 15 nM and 31 nM, respectively. These results will facilitate cryo-EM studies on GPCRs with interacting partners, using a tethering system that both activates the GPCR and maintains it in a concentrated form within a restricted, two-dimensional plane; and also will aid a wealth of mutational and lipid-dependent studies whereby the effect on coupling and GPCR-protein interactions of specific residues and lipid types can be directly measured.

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P305

NEUROTENSIN TYPE I RECEPTOR: IMPACT OF THE LIPID BILAYER ON THE STABILITY OF HELIX 8

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Neurotensin (NT) is a 13-amino-acid peptide, which plays a key role in the pathogenesis of Parkinson's disease, schizophrenia, antinociception, in hypothermia and in lung cancer progression. It is expressed throughout the central nervous system and in the gut, where it binds to at least three different neurotensin receptors (NTS): NTS1 and NTS2, which are class A G Protein Coupled Receptors (GPCRs), and NTS3, which belongs to the sortilin family. Most of the effects of neurotensin are NTS1-mediated, with the peptide acting as an agonist. Thus, providing atomistic insights on the structure and dynamics of NTS1 and its binding to neurotensin is of huge importance in the structural biology field.

Among the latest crystal structures deposited for NTS1[1,2] there is a dicrepancy regarding the existence[2] or not[1] of Helix 8 as a helical secondary structure motif, which is observed in most GPCRs structures reported so far. In addition, the functional role of Helix 8 on NTS1 function is still under dispute. Here, we have employed Molecular Dynamics simulations for the inactive state of NTS1 embedded in a membrane of different lipid compostions, in combination with EPR experiments, to provide a better understanding of the role of the lipid membrane on the stability of Helix 8 secondary structure. These results will be further validated by ssNMR experiments.

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P401

FRAGMENT-BASED LEAD DISCOVERY BASED ON ADENOSINE RECEPTOR CRYSTAL STRUCTURES AND HOMOLOGY MODELS

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G protein–coupled receptors (GPCRs) are intensely studied as drug targets and for their role in signaling. With the determination of the first crystal structures for GPCRs, the focus on structure-based ligand discovery has increased. Several high-resolution structures of the A_{2A} adenosine receptor (A_{2A}AR), which is a target for development of drugs against Parkinson's disease, have now been determined. The structures for the other three members of the family (A_1-, A_3-, and A_{2B}ARs) are still unknown, but the high sequence similarity to the A_{2A} subtype makes it possible to predict these using homology modeling. Examples of fragment-based lead discovery (FBLD) based on AR crystal structures and homology models will be presented.

We have identified several novel fragment ligands of the A_{2A}AR [1] based on molecular docking screens against a high-resolution crystal structure [2]. In a first step, we blindly predicted the results of an NMR-based fragment screen of 500 compounds [3] using docking, identifying four of the five ligands that were discovered experimentally. In addition, we also identified three false-negatives from the NMR screen. In a second step, we then prospectively screened a library of 328,000 commercially available fragments [4] and tested 22 top-ranked compounds experimentally. Fourteen of these compounds were found to be A_{2A}AR ligands in radioligand assays, which corresponds to a hit rate of 64%. Two of the identified fragments were optimized for affinity, but the resulting ligands were not selective for the A_{2A}AR subtype.

Unfortunately, structures for three ARs subtypes are still unknown. In these cases, structure-based screens are forced to rely on homology models. Access to high-resolution crystal structures of the A_{2A}AR makes it possible to generate models for the three closely related receptor subtypes, which could enable structure-based discovery of new compounds with specific selectivity profiles. However, it is not entirely clear if homology models can be used in FBLD. We have explored the use of biophysical and structure-based screening to identify selective A_3AR fragment ligands. These screens have enabled us to carry out comparisons of ligand discovery using modeled structures versus crystal structures. Our results also highlight opportunities and limitations of the use of crystal structures and homology models in drug discovery for this pharmaceutically interesting group of receptors.

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CHARACTERIZATION OF APOMORPHINE DERIVATES AS NOVEL SUBTYPE-SELECTIVE, HIGH-AFFINITY DOPAMINERGIC LIGANDS

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Dopamine receptors are G protein-coupled receptors (GPCRs), which are involved in a wide variety of physiological processes, for example voluntary movement, feeding, reward, attention, working memory and learning. However, abnormalities in dopaminergic system are associated with many different diseases. Therefore, dopamine receptors are targets for variety of drugs involved in diseases like schizophrenia, Parkinson's disease, depression and many others. Many currently used dopaminergic drugs have several activities, some of which can be seen as unwanted side-effects. Therefore, development of novel, subtype-selective ligands is in the focus of our research.

Apomorphine is one of the earliest pharmacological tools used to characterise dopamine receptors. Acting as a non-selective agonist, it is one of the few approved dopamine receptors' drugs. However, application of apomorphine has been limited by the above mentioned lack of selectivity among dopamine receptor subtypes. Therefore, the aim is to find compounds with higher subtype selectivity and affinity by modifying the structure of apomorphine. Binding activity and selectivity of 2-substituted, 3-substituted and 9,10-substituted apomorphine derivates, all together 25 compounds, was tested. For this, competitive radioligand binding assay was used and HEK293 cell lines stably expressing individual recombinant dopamine D_1, D_2L and D_3 receptors were created. [1]

The main purpose of this work was to discover novel, subtype-selective, high-affinity dopaminergic ligands. As a result, several compounds with nanomolar affinities for D_2 receptor were described. Also, some compounds with subnanomolar affinity and notable selectivity for D_3 receptor were identified. In summary, some promising functional groups, which improved binding properties of apomorphine derivates, were discovered.

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P403

GETTING BETTER AT PREDICTING BITTER

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Bitter taste is a significant factor in animal's choice of food. Animals avoid eating bitter food components, many of which are toxic. Nevertheless, it is known today that bitterness is not always noxious and that some of the bitter compounds have beneficial effects on health. Interestingly bitter taste receptors (a subfamily of GPCRs) are also expressed in many extraoral tissues and emerge as novel targets for therapeutic indications such as asthma and infection.

We aim to identify common properties of bitter compounds and to predict additional ones. To this end we established BitterDB [1] a database of bitter compounds, available at http://bitterdb.agri.huji.ac.il/bitterdb/, which currently includes over 600 compounds that were reported to be bitter for humans.

Recently we gathered structures of over 1500 compounds that are likely to be non-bitter. Chemoinformatic analysis of these sets suggests a chemical sub-spaces that bitter and non-bitter compounds occupy compared to random set of molecules (represented by the ChEBI dataset). We found sets of topological and ADME (absorption, distribution, metabolism, and excretion)/TOX descriptors that can partially distinguish between bitter and non-bitter compounds. Predictions of bitterness based on chemical structure and molecular descriptors are currently underway. In parallel, co-occurrence of compound name with the word "bitter" in PubMed publications was analyzed and resulted in a set of over 500 likely bitter compounds within clinically approved drugs and ChEBI molecules. These findings indicate the feasibility of predicting bitterness despite extreme variability among known bitter compounds and may facilitate potential repurposing of existing drugs for novel indications via their action on bitter taste receptors [2].

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IDENTIFICATION, DOCKING ANALYSIS AND VERIFICATION OF BINDING POCKET OF ALLATOSTATIN RECEPTOR (ALSTR) IN CARAUSIUS MOROSUS

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Mammalian somatostatin receptors (SSTR) have anti-proliferative effects in human tissues and this property made them valuable targets for cancer treatment [1]. In recent studies allatostatin receptor (AlstR), which has major roles in development and food response in insects [2], was found to be an ancient homolog of the somatostatin/galanin/opioid family of GPCRs [3]. In this study an AlstR has been identified from stick insect, *Carausius morosus*. According to phylogenetic analysis, the receptor was closely related to Drosophila Allatostatin Receptor C; so it was called as CamAlstR-C and was supposed to be activated by allatostatin C peptide (AST-C). In order to define the binding pocket of this receptor-ligand system and to propose an antagonism or mutation resulting in disruption of the system, homology modeling, ligand docking and molecular dynamics simulations were performed *in silico*. Binding pocket was found to consist of N-terminus together with the second and third extracellular loops (ECL). ECL3 region of the binding pocket was conserved within insect AlstRs. Binding with the ligand (AST-C) was verified via Atomic Force Microscopy (AFM) in nano scale. We proposed that the receptor-ligand interactions obtained from *in silico* predictions would be a reliable target region for analog and antagonist design studies. The residues selected from this conserved region were mutated via site-directed mutagenesis and their interactions with the peptide were compared in AFM experiments. Therefore the decrease in binding forces of mutant receptors supported the *in silico* predictions about the putative binding pocket.

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P405

DUALSTERIC ACTIVATION OF M2 MUSCARINIC ACETYLCHOLINE RECEPTORS INHIBITS CELL PROLIFERATION IN HUMAN GLIOBLASTOMA CELL LINES

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Muscarinic acetylcholine receptors (mAChRs) are expressed in several primary and metastatic tumours. ACh synthesized by the tumour cells can modulate cell proliferation by an autocrine mechanism which involves cholinergic receptors. Although a direct or indirect role of transduction pathways activated by cholinergic receptors has not yet been demonstrated, the inhibition of ACh synthesis as well as the release or use of cholinergic antagonists are able to counteract tumour cell growth and slow down the tumour progression in small cell lung carcinoma [1]. In mammary adenocarcinoma and melanoma cell lines, mAChRs can also modulate cell migration and angiogenesis, suggesting their involvement in the metastases formation [2]. The characterization of mAChR effects on more aggressive brain tumours is still poorly investigated. Glioblastomas are the most common brain tumours in humans. Recently, Tata et al. demonstrated that M2 receptor activation inhibits glioma cell growth and survival, suggesting that this receptor subtype may represent a new putative target for glioblastoma therapy [3,4]. Therefore, the identification of more selective ligands for M2 mAChRs may be of clinical significance. Here we report the results on the effects of the muscarinic orthosteric superagonist Iperoxo [5] and its related dualsteric agonists P-6-Iper and N-8-Iper [6]. Our data demonstrate that cell proliferation as well as cell survival of the U251 and U87 stable cell lines were unaffected by treatment with Iperoxo and P-6-Iper. Conversely, N-8-Iper decreased cell proliferation in a time and dose dependent manner. Similarly, N-8-Iper (100 μM) was also able to counteract cell proliferation in glioblastoma cancer stem cells (GB7) obtained from human biopsy. The antiproliferative effect shown by N-8-Iper was significantly counteracted by the selective M2 antagonist methoctramine (10^{-7} M), suggesting an actual contribution of the M2 selective activation.

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P406

CRYSTALLOGRAPHIC STRUCTURES GUIDE THE DISCOVERY OF SELECTIVE 5-HT$_{1B}$ RECEPTOR LIGANDS *VIA* VIRTUAL SCREENING

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G Protein-Coupled Receptors (GPCRs) are ubiquitous membrane proteins that comprise many relevant drug targets. Despite the recent advances in GPCR crystallography, the structure of the vast majority of GPCRs remains unknown. Consequently, the discovery of novel and selective GPCR ligands with structure-based methods often relies on the accurate computational prediction of GPCR structures.

The recent determination of crystallographic structures for the human serotonin 5-HT$_{1B}$ and 5-HT$_{2B}$ receptors bound to ergotamine revealed the molecular determinants of ligand binding to these related subtypes. We participated in the GPCR Dock 2013 assessment, where the molecular modeling community was challenged to predict these ligand-receptor complexes before the crystallographic coordinates were released.[1] For that purpose we employed a ligand-guided homology modeling approach that combines extensive experimental data and molecular docking screening.[2] Our best solutions were close to the experimental structures, being ranked as best and fifth-best predictions among all submitted 5-HT$_{1B}$ and 5-HT$_{2B}$ receptor complexes, respectively.

Later on we used the crystal structures of the 5-HT$_{1B}$ and 5-HT$_{2B}$ receptors to identify subtype-selective ligands. From docking screens of 1.3 million compounds, 22 molecules were predicted to be selective for the 5-HT$_{1B}$ receptor over the 5-HT$_{2B}$ subtype, a requirement for safe serotonergic drugs. Nine compounds were experimentally verified as 5-HT$_{1B}$ selective ligands with up to 300-fold higher affinities for this subtype, representing potential lead candidates against migraine.[3] Analysis of our best homology models of the two 5-HT receptors submitted to GPCR Dock 2013 revealed that despite their accuracy, they could not capture the critical features responsible for ligand selectivity. Our results demonstrate that structure-based screening is a fruitful approach to guide the discovery of selective ligands, and suggest novel methodological approaches for more accurate modeling of GPCR-drug complexes.

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P407

PHARMACOPHORE MODELING OF $GABA_B$ RECEPTOR LIGANDS – METHODOLOGY AND APPLICATION FOR VIRTUAL SCREENING

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γ-Aminobutyric acid B ($GABA_B$) receptors are postulated as potential therapeutic targets for the treatment of several brain disorders, including drug dependence. Apart from classical orthosteric ligands, the positive allosteric modulators (PAMs) have emerged as potential theraputic agents mimicking effects of agonists but having significantly reduced side-effects [1].

Due to the increasing numbers of published PAMs (74 structures in February 2014) some standard *in silico* approaches, such as pharmacophore modelling, may be utilized for the discovery of new active compounds. In this study, all known PAMs were hierarchicaly clustered using Canvas [2] with manual refinements to ensure proper chemotypes classification. Multiple hypotheses were developed for each cluster, employing the previously utilized approach [3]. After aplication of DUD-like [4] test set, one model per cluster was selected (according to Yourden's statistics value) to form the linear combination of pharmacophore models, i.e. the first, general pharmacophore hypothesis of $GABA_B$ PAMs.

This combination was applied as one of the steps in the virtual screening protocol reducing space of 5.3M of compounds from seven commericial databases to ~8K structures for further investigation.

Acknowledgments

The study was partially supported by the Polish-Norwegian Research Programme operated by the National Centre for Research and Development under the Norwegian Financial Mechanism 2009-2014 in the frame of Project PLATFORMex (Pol-Nor/198887/73/2013).

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P408

NOVEL POTENT SEROTONIN 5-HT$_7$ RECEPTOR LIGANDS: STRUCTURAL MODIFICATION TO IMPROVE PHARMACOKINETIC PROPERTIES AND IN SILICO PREDICTION MODEL

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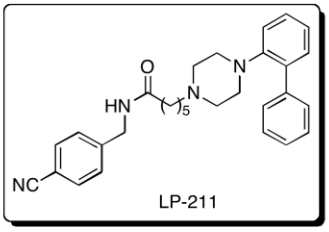
Serotonin 5-HT7 receptors (5-HT7Rs) are expressed in functionally relevant regions of the brain suggesting a role in many pathophysiological processes, such as depression, mood disorders, modulation of learning and memory [1]. During last years, our research group has been involved in the development of selective 5-HT7 receptor ligands and the most relevant outcome is represented by LP-211, a brain penetrant selective 5-HT7R agonist.

However, when LP-211 is administered *in vivo*, it is transformed into the main metabolite 1-(2-biphenyl)piperazine that retains affinity for the 5-HT7R [2].



LP-211

Since the pharmacology of this metabolite has been poorly explored, it is not known if its presence revert or attenuate the action of LP-211 *in vivo*. On such basis, the availability of novel 5-HT7R agonists with improved pharmacokinetic properties is desirable. Therefore, the scaffold of LP-211 has been manipulated to improve pharmakocinetic properties, leaving unchanged the structural features that are responsible for affinity, selectivity and agonistic properties towards the 5-HT7R. In silico model based on different molecular descriptors for the prediction of metabolic stability of the newly designed compounds has been developed using various machine learning techniques.

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P409

SIFT-GUIDED AGONIST/ANTAGONIST DIFFERENTIATION FOR BETA2-AR LIGANDS

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The results of ligand docking often fail to provide proper distinction between active and inactive compounds and between agonists and antagonists in particular, especially when only docking scores are considered. In order to predict the functional activity of a tested compound *in silico*, a docking protocol with Structural Interaction Fingerprint (SIFt, [1])-based automatically applied constraints, enabling evaluation of the functionality of ligands was developed. In the presented case of beta2-AR, annotated ligands were extracted from ChEMBL database (version 17) [2]. The initial docking was performed for 2 agonist- and 8 antagonist-bound crystal structures of beta2-AR. Calculation of SIFt profiles (as in previous research [3]) for those results enabled the distinction of agonist and antagonist binding modes, as well as highlighting the conformational differences in crystal structures of beta2-AR. The constraints were developed from the differences in those binding modes, and as shown by extensive cross-docking experiments (using Schrodinger's Glide [4]), expressed promising enhancement of docking performance for agonist-antagonist differentiation. A proper implementation of SIFt-based functional constraints may greatly improve virtual screening cascades by adding a highly desirable information about the potential functional activity of tested molecules.

Acknowledgments

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P410

INSIGHTS INTO THE TERMODYNAMIC PROPERTIES OF A 5HT1A LIGAND

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Serotonin (5-hydroxytryptamine; 5-HT) is a neurotransmitter and a vasoactive hormone synthesized from the amino acid tryptophan mainly in the enterochromaffin cells of the intestine and in the brain. It is a key mediator in the physiology of mood, vascular function and gastrointestinal motility. Based on biochemical and pharmacological criteria, serotonin receptors are classified into seven main receptor subtypes, 5-HT1–7. Of major pharmacotherapeutic importance are those designated 5-HT1, 5-HT2, 5-HT4, and 5-HT7, all of which are G-protein-coupled, whereas the 5-HT3 subtype represents a ligand-gated ion channel. The 5-HT1A receptor was the first cloned 5-HT receptor [1,2] suspected to be important to affective disorders. It couples to Gi/Go proteins, in most cells inhibiting adenylyl cyclase activation, reducing cAMP levels. [3,4]

This work expounds the thermodynamic properties of BTPN (4-[4-(3-benzo[b]thiophen-3-yl-3-oxopropylamino)piperidin-1-yl]benzonitrile) [5], a 5HT1A ligand. Receptor binding experiments with human 5HT1aR from cell culture showed that BTPN is a moderately high affinity ligand with Ki of about 10 nM at 30 degrees C. The binding experiments (in the presence of [3H]8-OH-DPAT; 8-Hydroxy- dipropylaminotetralin) at 0, 15, 25, 30 and 35 degrees C gave a shallow, negative slope van't Hoff plot, yielding a standard enthalpy of +28 kJ/mole. Using an Arrhenius-like expression, standard free energy was determined to be -50 kJ/mole with entropy from the Gibb's free energy expression of 261 J/mole. We also report a limited number of investigations into the mechanistic properties of BTPN as well as brief explorations of binding versus the beta adrenergic antagonist [3H]Dihydroalprenolol. We assume this information in the context of a 50 member series of structural relatives (including serotonin transporter; SERT inhibitors) of BTPN that will be useful to modelers in discovering better anti-depressant drugs.

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P411

NOVEL DERIVATIVES OF 2-AMIDOCYCLOHEX-1-ENE CARBOXYLIC ACID AS HCA_1, HCA_2, AND HCA_3 RECEPTOR AGONISTS

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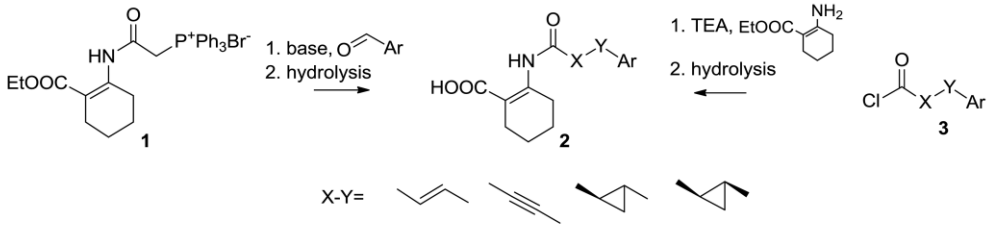
Hydroxy-carboxylic acid (HCA_1, HCA_2 and HCA_3) receptors are G-protein-coupled receptors which are expressed in adipocytes. HCA_2 and HCA_3 receptors are also expressed in a variety of immune cells. HCA_2 receptor is the supposed target of antidyslipidemic drug nicotinic acid. HCA receptors represent promising drug targets for metabolic disorders, inflammatory, immune and other diseases [1].

A variety of synthetic ligands for HCA receptors have been developed. The series of 2-propanamidocyclohex-1-enecarboxylic acid moiety containing compounds **2** (X-Y = $-CH_2CH_2-$) were designed at Merck as HCA_2 agonists and several compounds were identified as more active than nicotinic acid and with better pharmacokinetic properties [2, 3]. Because the incorporation of rigidity elements in the linker X-Y could help to pre-organize the molecule in a favorable bioactive conformation, we synthesized cyclohex-1-enecarboxylic acid derivatives **2** with linkers X-Y as well as various aryl groups (Ar) [4].



To evaluate the possible agonist properties of the synthesized compounds towards human HCA_1, HCA_2, and HCA_3 receptors, forskolin-stimulated cAMP accumulation assays were arranged. Our studies showed that incorporation of rigid linkers X-Y such as *E*-double bond or triple bond increase activity and selectivity of 2-naphtalenyl and 6-hydroxy-2-naphtalenyl derivatives. It was established that activity and selectivity is critically dependent on the aromatic part of the molecule.

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P412

STRUCTURAL INTERACTION PROFILES COMBINATION AS A METHOD FOR OPTIMIZATION OF ITS APPLICATION IN DOCKING RESULTS ANALYSIS – BETA-2 ADRENERGIC RECEPTOR CASE STUDY

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Prediction of spatial orientation of a molecule in a binding pocket of a given receptor and inferring on its basis about the potential activity of a particular compound still constitutes a very challenging task for computational part of drug design campaigns [1]. There are some approaches that enable automation of the procedure of ligand-protein complexes analysis, among which there is a combination of Structural Interaction Fingerprints with machine learning algorithms [2]. However, there still remains the problem of selection of proper set of models for docking studies – should it be just one receptor providing the best discrimination between actives and inactives or maybe using ensemble approach is better, as it is in case of ALiBERO [3].

The primary objective of the study was to optimize the number of homology models used for SIFTs profiles calculations on the basis of ligand-beta2 adrenergic receptor complexes. The results obtained for homology models of the receptor constructed on 9 different templates were also compared with docking performed with the use of crystal structures of the protein. The docking outcome was represented by Structural Interaction Fingerprint (SIFt) and for each ligand such representation was averaged over various models used for particular analysis (the number of models taken into account ranged from 3 to 20). Such data was then examined with the use of the Support Vector Machine algorithm to distinguish profiles belonging to active molecules from those that were characterizing inactive compounds. The analysis enabled determination of the optimal number of models that are recommended for use in this kind of study.

Acknowledgements

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P413

THE MULTICOMPONENT APPROACH TO GPCR MODULATION: ADENOSINE ANTAGONISTS BASED ON THE 4-AMINOPYRIMIDINE CHEMOTYPE

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Multicomponent reactions (MCR) have emerged as tailored synthetic methodologies in drug discovery [1], they provide access to large collections of functional, skeletal and stereochemically diverse scaffolds in a time- and cost-effective manner [2]. In the frame of a structure-guided MCR-driven program [3] we herein document a novel multicomponent pyrimidine synthesis (Figure 1) enabling to decorate, according to the specific requirements of the different adenosine receptor subtypes (A_1, A_{2A}, A_{2B}, A_3), the privileged 4-aminopyrimidine scaffold; thus generating novel series of structurally simple, potent and highly selective adenosine receptor antagonists.

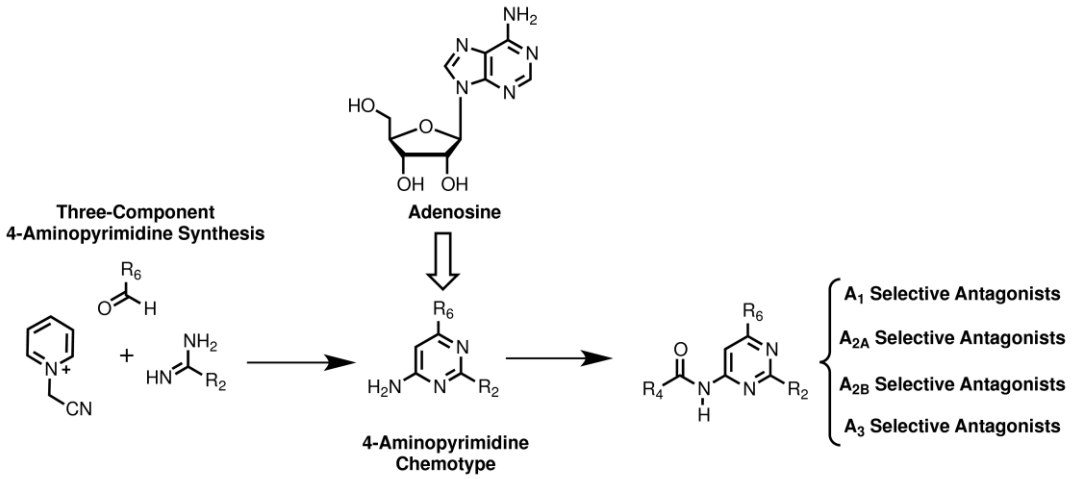

Figure 1. Design and synthetic strategy employed during the assembly of 4-aminopyrimidine ligands

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BINDING MODE PREDICTION OF TRICYCLIC XANTHINE DERIVATIVES AT THE ORTHOSTERIC LIGAND-BINDING SITE OF A_{2A} AND A_{2B} ADENOSINE RECEPTORS

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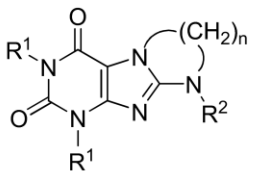
Xanthines are widely known group of purine-based alkaloids that exhibit a variety of physiological effects, such as positive inotropic and chronotropic effects on the heart, decreased airway resistance in the lung and respiratory stimulation as well as significant behavioral effects on measures of locomotor activity, schedule-controlled behavior, drug self-administration and learning and memory. It is suggested that the psychostimulant and cardiac effects of xanthines are connected principally with the inhibition of adenosine receptors, located both in heart and brain.

In recent years many studies have been conducted in the direction of the potential use of synthetic xanthine derivatives, including polycyclic fused ring systems, as potent antagonists at various adenosine receptors subtypes. Here we focus on a series of tricyclic imidazo-, pyrimido- and diazepinopurine-2,6-diones, obtained and pharmacologically characterized by our groups [1, 2]:



R^1 = methyl, ethyl, propyl, butyl
R^2 = alkyl, cycloalkyl, aryl, aryl-alkyl
n = 2, 3, 4

To predict possible binding modes of the described compounds inside the binding pocket of the human A_{2A} and A_{2B} adenosine receptors, molecular modeling and docking methods have been applied. A large series of tricyclic xanthine derivatives with an additional five-, six- or seven- membered ring fused to the *f*-bond of the 2,6-purinedione system was docked into the 3EML crystal structure and two homology models of A_{2A} and A_{2B} receptors built on the basis of 3EML template. To improve docking results, the induced fit docking protocol allowing reorientation of side chains within the binding site, was also applied. The calculated docking poses of ligands inside the binding pocket and putative ligand-protein interactions for both A_{2A} and A_{2B} were compared and discussed.

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COMBINED IN SILICO APPROACHES FOR THE SELECTION OF DUAL CCR5/CXCR4 INHIBITORS

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CCR5 and CXCR4 are the two most important coreceptors for HIV entry. Inhibitors targeting both CCR5 and CXCR4 may be highly important for inhibiting viral transmission and replication and delaying the onset and progression of the disease. Recently, we demonstrated strong correlation between the electron-ion interaction potential (EIIP) and the average quasi-valence number (AQVN), the molecular descriptors determining long-range intermolecular interactions, and anti-HIV activity of organic molecules [1]. The overlapping EIIP/AQVN domain of CCR5 and CXCR4 suggest possibility to select dual CCR5/CXCR4 inhibitors. In this study we performed in silico screening with the EIIP/AQVN filter of the 3ML virtual library composed of three million compounds and selected 300 compounds that were further filtered by means of 3D QSAR. Using clinically confirmed anti-HIV agents as references, the compounds were filtered through PCA model of GRID descriptors in Pentacle software [2-4]. We have identified 15 candidate compounds for CCR5/CXCR4 inhibitors that would be synthesized with combinatorial synthetic methodology and further evaluated in biological assays.

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P416

HYDANTOIN DERIVATIVES AS SELECTIVE SEROTONIN 5-HT$_7$ RECEPTOR LIGANDS

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Recent lines of evidence indicates relevant role of 5-HT$_7$ receptor in thermoregulation process, memory and learning, hormonal regulation and also in hippocampus activity, circadian rhythm and mechanism of depression. Already synthesized ligands of this receptor, despite the high affinity, cause side effects as a consequence of lack of selectivity, which is still very challenging to achieve among GPCRs [1]. Our studies allowed to find first selective 5-HT$_7$R ligand among aminoalkyl derivatives of hydantoin [2]. Although the structure contains arylpiperazine fragment which is known to interact with many GPCRs, putatively geometry of hydantoin is relevant to achieve selectivity. However, obtained compound was an orphan, that is why synthesis of its analogues was vital to identify structural fragments which are responsible for selectivity with simultaneous maintaining high affinity to 5-HT$_7$. For this moment, about 20 new compounds have been obtained. Their general structure is presented in Fig.1:

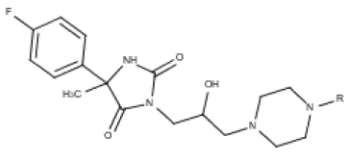

Fig. 1

The new compounds were synthesized in three-step pathway (Bucherer-Berg condensation, Mitsunobu reaction, condensation using microwave irradiation). Among this group of compounds, there are highly active compounds (3 nm < K_i < 234 nm). It turned up that when R-group is biphenylmethyl moiety, the highest selectivity regards to 5-HT$_{1A}$ is observed, moreover all the synthesized derivatives show selectivity regards to D_2R. Organic synthesis is supported with molecular modeling techniques: homology modeling, fit induced docking and machine learning, which allow us to study protein-ligand interactions and predict selectivity. Our studies lead to choose new selective 5-HT$_7$ ligand among hydantoin derivatives, which will be ready for biofunctional and *in vivo* studies. It may have significant meaning in future CNS diseases therapy. Partly supported by Polish program K/ZDS/003323.

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P417

EXPERT CURATED INFORMATION ON GPCRS IN THE IUPHAR/BPS GUIDE TO PHARMACOLOGY

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G protein-coupled receptors (GPCRs) are the largest family of human drug targets representing ~19% of the targets of current drugs and many more in clinical trials [1]. We have developed the IUPHAR/BPS Guide to PHARMACOLOGY portal (the new home of the IUPHAR Database) to provide free access to curated information on important pharmacological targets and the substances that act on them [2]. The database includes data on 394 GPCRs including 130 "orphans" without confirmed endogenous ligands. This includes the recent addition of 33 adhesion class GPCRs. Development of the database is overseen by the International Union of Basic and Clinical Pharmacology (IUPHAR) Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR). Expert subcommittees for each GPCR family provide recommendations on receptor nomenclature and peer-reviewed summaries of the literature covering properties such as function, pharmacology, signalling mechanism, important variants, available assay systems and mouse knockout phenotypes. Ligands are annotated with their chemical structures, or sequences and post-translational modifications for peptides. Links are provided to other online databases including GPCRDB, UniProt, Ensembl and PubChem. Current work includes identifying the mechanism of action for approved drugs treating human diseases, mapping them to their primary targets and curating supporting data in the literature (*e.g.* K_i, K_d, IC_{50}). In some cases we also annotate data-supported polypharmacology where interactions of comparable *in vitro* potencies against multiple targets have been published. The database is available online at http://www.guidetopharmacology.org.

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P418

GSA-10, LEAD OF A NEW CLASS OF NON CANONICAL POSITIVE MODULATOR OF SMOOTHENED

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The Hedgehog (Hh) signaling pathway regulates stem cell maintenance and repair in adult tissues[1]. Hh proteins modulate electrical activities of mature neurons and stimulation of the Hh pathway has shown therapeutic efficacy in models of Parkinson disease, diabetic neuropathy, demyelination and myocardial ischemia suggesting that small-molecule agonists of the Hh pathway may have therapeutic interest. Smoothened (Smo), a seven-transmembrane protein belonging to the G protein-coupled receptor superfamily, is the major transduction component of this pathway. High-throughput screening of chemical libraries has led to the identification of the Smo reference agonists SAG, a chlorobenzothiophene and purmorphamine, a purine derivative as well as related molecules. These molecules have been used for modulating embryonic stem cells and adult neural precursors cells but none has reached clinical trials[2].

Here, we have generated and validated a pharmacophoric model for Smo agonists and used this model for the virtual screening of a library of commercially available compounds. Among the 20 top scoring ligands, we have identified and characterized a novel quinolinecarboxamide derivative (GSA-10) as a Smo agonist[3]. Using pharmacological, biochemical and molecular approaches, we provide compelling evidence that GSA-10 acts at Smo to promote the differentiation of multipotent mesenchymal progenitor cells into osteoblasts. However, this molecule does not display the hallmarks of reference Smo agonists. Remarkably, GSA-10 does not recognize the classical bodipy-cyclopamine binding site, induces neither Gli-dependent reporter gene transcription nor cerebellar granule cell proliferation, and it does not regulate the subcellular localization of Smo at the primary cilium. Moreover, we observed that cholera toxin and forskolin, two known activators of adenylate cyclase, are positive and negative regulators of GSA-10 and SAG-mediated cell differentiation, respectively. Thus, GSA-10 belongs to a novel class of Smo agonists and should be helpful for dissecting Hh mechanism of action with important implications in physiology and in therapeutic[4].

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PG01

A NEW CRYSTAL STRUCTURE FRAGMENT-BASED PHARMACOPHORE METHOD FOR GPCRS

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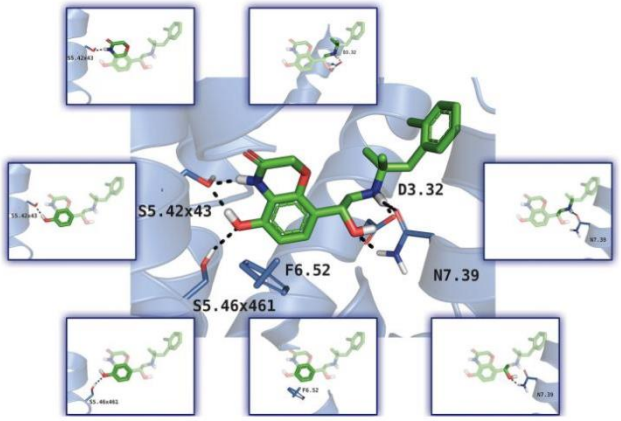

A pharmacophore model is a generalized description of a compound defined as a spatial orientation of different features of a molecule(s) [1]. Such a model can describe a vast number of chemical compounds with only a handful of common features, and thus is broadly used in virtual screening to identify novel ligands.

We present a new method for building pharmacophore models for GPCRs that combines the ligand- and target-based methods by extracting interacting ligand moiety – receptor residue pairs from crystal structures complexes [2]. Our library covers 250 such fragments and 29 residue positions (Ballesteros-Weinstein numbering [3]) within the binding pockets of class A GPCRs. The library fragments are recombined and inferred to construct pharmacophore models for novel targets, for which no (homologous) crystal structures or ligands are known. The method has a significant screening potential – supported by a case study on histamine H1 and H3 receptors yielded a hit rate of 14% and best potency of 660 nanomolar. In addition, the side chains of residues extracted from the crystal structures constitute a library of position-specific rotamers, that can be applied for refinement of homology models.

The current fragment library makes it possible to target ~47% of the class A GPCRs with at least four-element pharmacophores. The fragment library, along with an online tool aligning them with a receptor structure is available on the GPCRDB tools website (tools.gpcr.org).

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PG02

GPCRDB: GPCR DATA, DIAGRAMS AND TOOLS

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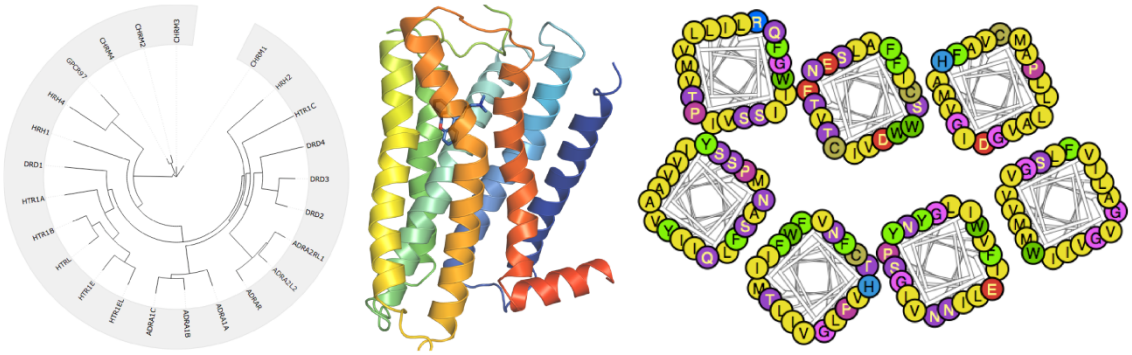

GPCRDB (gpcr.org/7tm) has been a popular resource for the G protein-coupled receptors community for the past 20 years and obtained more than 1000 citations [1-5]. GPCRDB contains experimental data on crystal structures, mutations and oligomers, as well as computationally derived sequence alignments and homology models. The latest release has added user-friendly web browser tools and diagrams for downloaded for publication (tools.gpcr.org).

NEW	**Diagrams**	• Interactive residue snake- and helix box plots • Phylogenetic trees based on any subsequence
GPCRDB	**Data**	• Structure-based sequence alignments and 3D models • Sequence conservation statistics for alignments
FEATURE		• Generic residue numbering of sequences and structures
HIGHLIGHTS	**Tools**	• Crystal structure browser with annotations • Ligand off-target prediction by binding sequence motif search • Receptor similarities based on any subsequence

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PG03

GENERIC G PROTEIN-COUPLED RECEPTOR RESIDUE NUMBERING - ALIGNING TOPOLOGY MAPS WHILE MINDING THE GAPS

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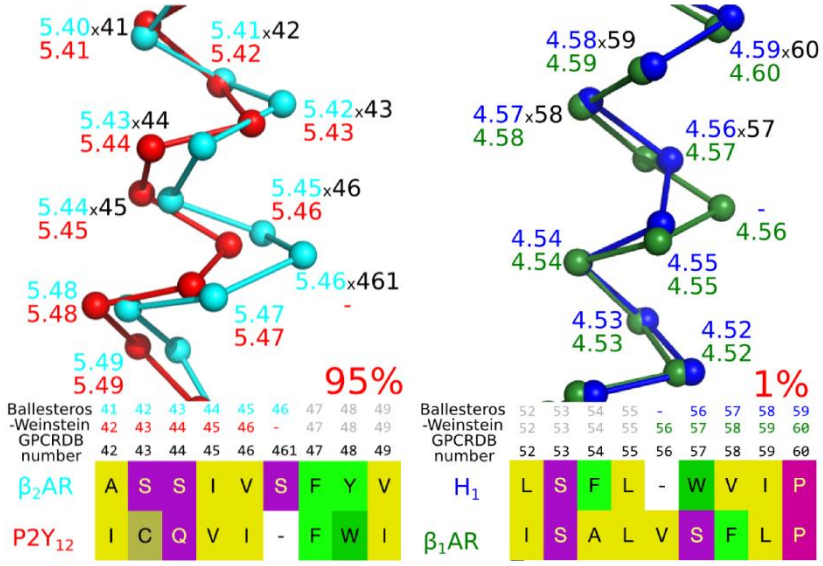

Generic GPCR residue numbers denote the corresponding residues within receptors of interest to facilitate comparisons of e.g. mutational effects, ligand interactions and structural motifs. Their utility is illustrated by the more than 1100 citations for the most commonly used scheme of Ballesteros and Weinstein [1] for class A GPCRs. The first crystal structures for the other classes B, C and F now put the spotlight on how to assign residue numbers within and across these classes. Furthermore, as we entered the structural era, we found that GPCR helices contain frequent bulges and constrictions that offset the generic residue numbers [2].

Here, we provide recommendations for the use of generic GPCR numbering schemes. Moreover, we introduce a complementary structure-based scheme to take proper care of the helical bulges and constrictions. Finally, GPCRDB [3] has been equipped with user-friendly retrieval of the first comprehensive crystal structure-based sequence alignments and tools to assign generic residue numbers to any receptor sequence or structure.

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