Summary of the « 5th annual meeting of the GDR

This year, the 5th GDR3545 annual meeting took place in Tours on November 22-24th of 2016 at the "hôtel de l'Univers". 188 scientists including 17 foreign scientists from 8 different countries (USA, India, China, Italy, Germany, Canada, Spain, Switzerland and UK) participated to this annual meeting organized by Dr Pascale Crépieux (BIOS team, PRC, INRA) and the GDR members from Tours (teams BIOS, DRuGS, NMR and PRC unit). During this meeting we had 74 posters, 28 oral presentations (7 from GDR members, 8 from invited foreigner scientists, 2 French speakers outside the GDR and 11 short talks given by young scientists) and 9 short presentations from sponsors. Furthermore, similarly to previous years, the meeting was preceded by the young scientist workshop (November 21-22nd, 2016), which in this edition focused on "Building GPCR signalization networks. Example of the FSH receptor". Additionally, the meeting this year was followed by the workshop "Antibodies Targeting GPCRs, Recent Advances and Therapeutic Challenges", organized by Dr Mohammed Ayoub, Dr Eric Reiter and LE STUDIUM (November 24-25th, 2016).

Oral presentations

GPCR structures and dynamics

The meeting started with a session focused on the structure and the dynamics of GPCRs. Dr. Beili Wu from the Shangai Institute of Materia Medica presented interesting results on P2Y1R and P2Y12R, two receptors involved in the regulation of platelet activation and thrombus formation. Her team reported the crystal structure of the two P2Y receptors bound with agonist or antagonist and the binding of a non-nucleotide antagonist BTPU in a pocket located at the external interface of the P2Y1R receptor. This is the first report, published in 2015 in Nature (Zhang D et al., Nature, 2015), on a bound ligand crystallized outside the helical bundle pocket of a GPCR, and this information may have a huge impact on drug design.

Then, Dr Arun Shukla from the Indian Institute of Technology of Kanpur, India, presented his work on GPCR-β-arrestin interaction. Using specific antibodies targeting activated β-arrestin, he and his collaborators succeed to obtain the structure of the GPCR-G protein-β-arrestin complex using X-Ray crystallography, 3D reconstruction and single molecule electron microscopy. These data were recently published in Cell (Thomsen et al., Cell 2016).

The third invited speaker was Dr Didier Rognan from UMR 7200 CNRS, Université de Strasbourg, who presented results from an *in silico* screening to identify compounds targeting the druggable pocket of smoothened (SMO) homodimers interface. SMO is a strategic target for drug design, as it is involved in embryonic development and tumour initiation and progression. Using various in vivo tests, Dr Didier Rognan and collaborators validated a new SMO inhibitor DR-90, displaying a new mechanism of action and binding mode.

This session continued with the presentation of the selected young scientist. Dr. Lydia Caro, a research engineer (UMR7242 Unit, Illkirch), who showed by double electron resonance spectroscopy that a single amino acid substitution, detected in mutants related to congenital stationary night blindness, disturbs the conformational equilibrium of rhodopsin. The two post-doctoral fellows, Dr Julie Quoyer (Institut Cochin, Paris) and Dr Thor C. Møller (Institut de Génomique Fonctionnelle, Montpellier) presented their work on GPCR oligomerization in living cells using total internal reflection fluorescence microscopy and two photon fluctuation microscopy, or combined HTRF and nanobodies approaches respectively.

GPCR signalling

This session focused on one of the main downstream GPCRs signalling pathways, β-arrestins. Dr Mark Scott from Institut Cochin (Paris) focused on the PTEN/PI3K/AKT signalling. Using a double hybrid system, he found that the tumor suppressor PTEN is a partner of β-arrestin and thus inhibits this pathway. They recently developed an intra-molecular Bioluminescence Resonance Energy Transfer (BRET) sensor to detect PTEN conformational rearrangement to better understand this pathway. The dual role of β-arrestins was also the main topic of Dr Stéphane Dalle presentation, from Institut de Génomique Fonctionnelle (Montpellier), in the regulation of pancreatic β cell function. Whereas β-arrestin 2 regulates the glucagon-like peptide-1 (GLP-1) receptor desensibilisation, β-arrestin 1 mediates GLP-1 signalling, insulin secretion and apoptotic effect.
The session ended with the presentation of the young scientist, Dr Revu Ann Alexander (Institut Cochin, Paris) on FAK, a scaffold and kinase protein, and its interaction with β-arrestins 1 and 2. She also observed altered FAK localization and phosphorylation in β-arrestins knock-down cells. Overall, these works confirm that GPCR-β-arrestins signalling are critical in human diseases such as cancer.

GPCR in physiopathology

This third section provided an integrative point of view by connecting GPCR modulation to physiological or pathological phenotypes. Dr Bice Chini (Institute of Neuroscience, Milan, Italy) presented her work on the molecular mechanisms underlying the autistic-like symptoms in mice lacking the oxytocin receptor (Oxtr). Her group identified the chloride transporter, KCC2, as a relevant target of Oxtr in the control of neuronal excitation-inhibition balance. Moreover, they developed a bivalent ligand (two oxytocins bound) that relieves autistic-like symptoms in mice (Leonzino et al., Cell reports 2016; Busnelli et al., J Med Chem 2016). The next speaker, Dr Bernard Masri (I2MC, Toulouse), showed that protamine, an anti-angiogenic drug already used in clinic is a ligand of the apelin receptor by cell-based fluorescent screening assay, acting as an antagonist of this receptor. Dr Liu, from the Huazhong University of Science and Technology (Wuhan, China), main interest are the dynamics of GPCR dimerization, especially class C GPCR (mGluRs and GABAB). He showed that GABAB receptor transactivates IGF-1 receptor and proposed a link between GABA signalling and longevity in worms (Chun et al., Nature Communication 2015). Dr Françoise Bachelerie (Paris-Sud University) described how the chemokine CXCL12 can trigger different signalling pathways depending on which receptor is activated, and how dysregulation of these signalling pathways increase susceptibility to develop HPV pathology. The last invited speaker of this session, Dr Hélène Castel (University Rouen-Normandie) studies the urotensin receptor, focusing on biased signalling. Biased synthetic ligands display differential effects on angiogenesis and tumor growth, properties to be further explored for drug development approaches. In this session, among the selected short talk from the young scientist, Joyce Koenen (University Paris-Sud) focused on the effect of CXCL12 on CXCR4 receptor involved in haematopoiesis. Finally, Florian Rebeillard (University Paris-Descartes) closed this section on the orphan receptor, GPR88, which displays nuclear localization in neurons and interacts with nuclear partner proteins, as evidenced by data obtained in yeast two-hybrid screening and PLA assays.

Drug Discovery targeting GPCR

The first talk of the 4th section was given by Dr Lidija Covic (Tifts Medical Center, Boston, USA) on pepducins, lipopeptides that can modulate GPCR function via their intracellular interfaces, and showed an example with PAR receptors in artery and liver diseases (Shearer et al., JBC 2016). Dr Francine Acher (University Paris-Descartes) presented the characterization of two novel and selective compounds for mGluR4 receptor that bind to a chloride binding site, which is adjacent to the orthosteric site. The relevance of these compounds was evidenced in inflammation and neuropathic pain rodent models (Zussy et al., Mol Psychiatry 2016). The selected short talk from Vanessa Hoguet (Pasteur Institute, Lille) focused on the bile acid receptor TGR5 and the putative beneficial role of specific compounds developed to activate this receptor exclusively in the intestine to treat type 2 diabetes. Raphaëlle Quillet (University of Strasbourg) identified and characterized a new molecule which binds to NPFF1R, a member of the RF-amide neuropeptide GPCRs, and modulates hyperalgesia. Dr Christiane Mendre (IGF, Montpellier) works on the development and characterization of two new ligands of the V2 vasopressin receptor, one chemically synthesized and one snake toxin, which are promising tools to treat renal diseases. Dr Tobias Langenhan (Würzburg University, Germany) investigates the mechanisms of adhesion GPCRs underlying mechano-sensory responses, using Drosophila as a model. The young scientist Lucie Esteoulle (University of Strasbourg) closed this section on the development of fluorocarbon-conjugated peptides which are metabolically more stable, increasing the plasmatic half-life of peptides. The proof of concept of this innovative strategy was performed using apelin 17 peptide.

Antibodies as tools to analyse GPCR

The last section of the meeting started with the presentation of Dr Francisco Ciruela (University of Barcelona, Spain), who discussed the different techniques used to detect GPCRs oligomerization in native tissues, such as in situ antibody-based proximity ligation assay and α-screen technologies. The next speaker, Dr Didier Boquet (CEA, Gif-sur-Yvette) developed specific antibodies targeting specifically endothelin B receptors on melanoma cancer cells. The work presented by Michaël Mathieu (IGF, Montpellier) focused on the characterization of conformational-specific nanobodies against mGlu receptor 2 as potent tools to detect receptor activation in living cells. The mGluR2 was also discussed in

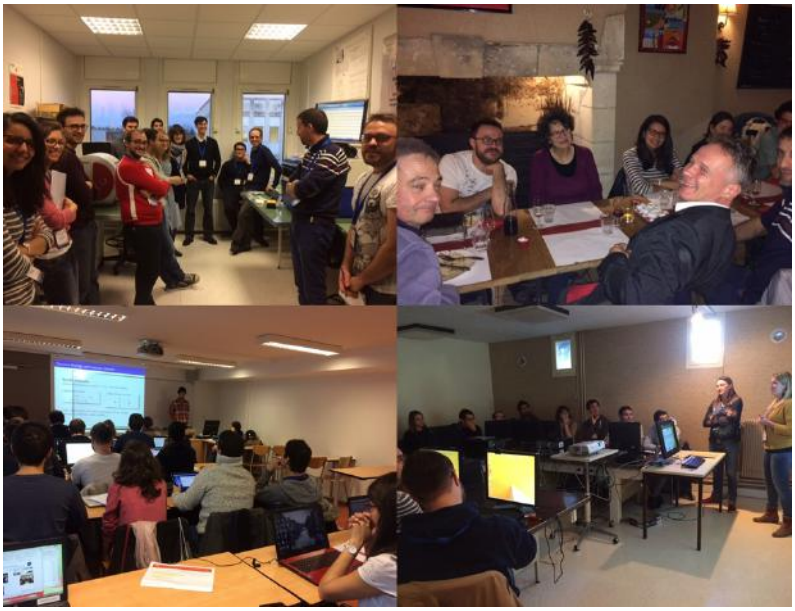
the presentation of Samy Murat (IGF, Montpellier), focusing on its dimerization with the serotonin 5-HT2A receptor, which leads to transactivation of mGluR2. Dr Rajan Digue from the Indian Institute of Science (Bangalore) closed the section by presenting his data on antibodies against the large N-terminal domain of glycoprotein hormones receptors, demonstrating that antibodies binding to glycoprotein binding site or the hinge region provide new insights on the activation-dependent conformational changes of the receptors.

Poster prizes

This year 74 posters were presented at the annual meeting over the three days. Similarly to the previous years, the best poster presentations were awarded with 4 poster prizes of 500€ each sponsored by the GDR (2 prizes, one for a PhD student and one for a postdoctoral fellow), Esteve Foundation (for a PhD student) and Interchim (for one Engineer). The committee judging the posters from PhD students was composed of Dr Masimiliano Beltramo, Dr Jacques Pantel and Dr Esther Kellenberger. Dr Julie Le Merrer, Dr Dominique Bonnet and Dr Xavier Iturrioz judged the postdocs and Engineers-technicians presentations. The Engineer Marine Luka (Paris), the postdoctoral fellow Dr Julien Diharce (Orléans) and the two PhD students Charlène Faye (Chatenay Malabry) and Alexandre Mutel (Rouen) were awarded.

Satellite workshop

Before the actual annual meeting, the 4th Workshop "Building GPCR signalisation networks. Example of the FSH receptor" organized by the young scientists committee and local committee took place on November 21st in the afternoon at the Unit Physiologie de la Reproduction et des Comportements, in Nouzilly and, on the next morning, November 22nd, at the Université François Rabelais de Tours. This workshop proposed practical and theoretical courses to 19 young postdoc and PhD students, an initiation to build GPCR signalling networks around the example of the FSH receptor. After a presentation of the sponsor Esteve Foundation by Felix Bosch, the training was dedicated to data acquisition and building GPCR networks with 3 practical exercises (Ingenuity Pathway analyses of RNA sequencing data, Interferometry and Living cell data acquisition of biased agonism using BRET), followed by a round table for discussion. The second morning session focused on dynamic molecular modelling of GPCR networks with Cell designer and Copasi softwares.



Sponsors presentations

This year, nine sponsors presented their products related to new tools and approaches to study GPCRs: the HTRF approach from Cisbio (Pauline Scholler); fluorescent GPCR signalling biosensors (Valery Savitsky from TEBU-Bio); detection of calcium signalling and β-arrestin recruitment (Thierry Calmels from Bioproject-Biotech); the generation of antibodies targeting GPCRs (Bruno Pitard from In-Cell-Art and Jean Chistophe Rain from Hybrigenics,); purification of GPCRs in cell-free system for screening approaches (Bruno Tillier from (Synthelis); purification of GPCRs, with the MT1 melatonin receptor as an example (Sebastien Igonet from Calixar); nanobodies technology (Oualid Sbai from Caminnov) and more general products related to GPCR study (Olivier Almeras from Interchim and Elodie Kara from Repropharm).

Conclusions:

The young scientist workshop was successful with around 20 participants and will be repeated next year. In general the young scientist committee is very active in the GDR providing four newsletters a year and organizing different GDR events. Furthermore, many new members were just recruited this year. This 5th edition of the annual GDR 3545 meeting was a great success, with excellent talks in the GPCR field and also selected presentation from young scientists, in an exciting environment to exchange and establish collaborations between more than 50 participating teams. We hope to see you again at the 6th annual meeting, which will be held on November 8-10th, 2017, in Paris.