JCI 2022
May, Monday 23rd and Tuesday 24th

Organized by:

BSC : Biotechnologie et signalisation cellulaire
CAMB : Laboratoire de conception et application de molécules bioactives
LBP : Laboratoire de bioimagerie et pathologies
LIT : Laboratoire d'innovation thérapeutique
ICube : Laboratoire des sciences de l'ingénieur, de l'informatique et de l'imagerie
IGBMC : Institut de génétique et de biologie moléculaire et cellulaire
PCBIS : Plateforme de chimie biologique intégrative de Strasbourg

Sponsors:

Posters

Characterization of new mouse models to study and treat a rare neurological and progressive disease

Victorine Artot[1234], Nicolas Zinter[1234], Wojciech Krezel[1234]

1-Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), 67404 Illkirch, France
2-Inserm, U 1258, 67404 Illkirch, France
3-CNRS UMR 7104, 67404 Illkirch, France
4-Université de Strasbourg, 67000, France

Retinoic acid is an indispensable mediator of neurodevelopment, although the associated regulatory mechanisms are not fully understood. Its signalling is transduced by retinoic acid receptors, which act as ligand-regulated transcription factors. *De novo* point mutations of the retinoic acid receptor β (RARb) cause rare syndromic disease called Microphthalmia type 12 (MCOPS12). Patients in addition small eye phenotype display also progressive motor abnormalities and cognitive deficits. In vitro studies revealed that such point mutations cause either a gain-of-function, loss-of-function, or dominant-negative effect and are associated with similar symptoms.

Much of what we know about RARb functions in the brain comes from analyses of $Rarb^{-/-}$ mice. Such studies revealed that RARb plays role in development and neuroprotection of striatum, a brain structure involved in motor control and cognitive functions.

Two new mouse models carrying either a gain-of-function mutation (p.Arg394Cys) or negative dominant form of RARb (p.Leu402Pro) have been generated. In order to characterize these models, we used distinct tests addressing motor functions. We were able to demonstrate that $Rarb^{R394C/+}$ and $Rarb^{L402P/+}$ mice have an identical motor phenotype, and the deficits described in patients. These models are valid for the study of motor disorders in MCOPS12 disease and our preliminary data suggest potential mechanisms of brain dysfunction.

Authors wanted to address many thanks to the Cure MCOPS12 foundation for their financial support. They also thank Dr. Jacques Michaud for providing animal models, the ICS behavioral facility and Alexis Simon and Peggy Mellul for animal care.

Role of Retinoic Acid Receptorβ Signaling in cell fate determination in health and disease

Ann-Kathrin Berger[1234]; Nicolas Zinter[1234]; Wojciech Krezel[1234*]

1-Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), 67404, Illkirch, France
2 Inserm, U 1258, 67404 Illkirch, France
3 CNRS UMR 7104, 67404 Illkirch, France
4 Université de Strasbourg, 67, France
** Corresponding Author (krezel@igbmc.fr)*

Retinoic acid (RA), an active form of vitamin A, is indispensable for the development and function of a wide array of organs in vertebrates. Loss or gain of RA signaling leads to abnormal development indicating a necessity to strictly control RA bioavailability. In target cells, RA binds to heterodimeric receptor complexes composed of a retinoic acid receptor (RAR) and a retinoid X receptor (RXR), which act as ligand-activated transcription factors and modulate target gene transcription by binding to DNA motifs and recruiting corepressors and coactivators.

De novo mutations in the RARb gene, which codes for the retinoic acid receptor beta (RARb), cause a progressive form of neurological disorder with sever motor and cognitive symptoms. Transfection studies indicate that these *de novo* mutations increase RARb transcriptional activity, or lead to dominant negative effect. We have generated a mouse model of this diseases which recapitulates behavioral abnormalities observed in patients. Preliminary data suggest possible role of RARb in control of neural cell fate. To address this question, we have generated a mouse model dedicated to studying role of RARb in cell fate determination in mice carrying point mutations causing disease and their wild type counterparts. This model and preliminary data will be described during poster presentation.

Authors wanted to address many thanks to ANR RAinRARE for their financial support, to Alexis Simon and Peggy Mellul for animal care, to Marie-Christine Birling and Genetic Engineering and Design ICS facility for mouse model generation, and to the IGBMC photonic microscopy imaging platform.

Design, synthesis and biological evaluation of new fluorinated compounds derived from Spexin for the potential treatment of pain

Yann Berthomé[1], Rosine Fellmann-Clauss[2], Claire Marsol[3], Maria Vittoria Spanedda[4], Line Bourel[4], Frédéric Simonin[5], Dominique Bonnet[3]

1 : Laboratoire d'Innovation Thérapeutique, Institut du Médicament de Strasbourg, UMR7200, CNRS/Université de Strasbourg
2 : Biotechnologie et Signalisation Cellulaire, UMR7242 CNRS/Université de Strasbourg
3 : Laboratoire d'Innovation Thérapeutique, Institut du Médicament de Strasbourg, UMR7200 CNRS/Université de Strasbourg
4 : Laboratoire de conception et application de molécules bioactives, UMR 7021 CNRS/Université de Strasbourg
5 : Biotechnologie et Signalisation Cellulaire, UMR7242 CNRS/Université de Strasbourg

---

Chronic pain is a major public health issue, which has a huge impact on society. Approximately 40% adults worldwide suffer from chronic pain and its total cost has been estimated at 560-635 billion dollars/year in the United States(1). Even if research progresses and new targets appear for treating acute and chronic pain, opiates still represent the gold standard analgesics. However, opioid treatments induce several adverse side effects among which analgesic tolerance and opioid-induced hyperalgesia (OIH) are of major importance. Hence, there is an urgent need to develop novel analgesics with fewer side effects. In this context, a promising neuropeptide, Spexin (SPX), has been discovered using bioinformatic methods(2) and was recently deorphanized. Ligand-receptor interaction studies have showed that SPX specifically activates two subtypes of the G protein-coupled receptor GalaninR2 and R3 (GALR2/3)(3). Moreover, SPX was found to induce a dose-dependent and opioid-independent analgesic response when centrally injected in rats(4). To increase the metabolic stability and the in vivo efficacy of biologically active peptides, our team has recently developed a new approach named FluoroPEP. This is based on the introduction of a perfluorinated carbon chain (F-chain) onto peptides to induce their self-organization as fluoropeptide counterparts in aqueous solution, resulting in the protection of the native peptides from enzymatic degradation (Figure 1).

This strategy was first validated on apelin GPCR peptide(5). In this communication, we will present the extension of the FluoroPEP approach to SPX, especially the design, the synthesis and the biological evaluations of the first fluorospexins to study the implication of GPCR GALR2 in pain modulation and to develop potential novel agents for the treatment of pain.

1. Gaskin, D. J.; et al. The Journal of Pain 2012, 13, 715–724. DOI: 10.1016/j.jpain.2012.03.009
2. Mirabeau, O.; et al. Genome Research 2007, 17, 320. DOI: 10.1101/gr.5755407
3. Kim, D.-K.; et al. Endocrinology 2014, 155, 1864. DOI: 10.1210/en.2013-2106
4. Toll, L.; et al. FASEBJ., 2012, 26, 947-54. DOI: 10.1096/fj.11-192831
5. Llorens Cortes, C.; Bonnet, D.; et al. Nat. Commun. 2021, 12, 305. DOI: 10.1038/s41467-020-20560-y

PDZ–PBM interactome: Visualization of features in protein lists and machine learning affinity predictions

Goran Bich, Yves Nominé, Gilles Travé

Institut de Génétique et de Biologie Moléculaire et Cellulaire
Université de Strasbourg, Centre National de la Recherche Scientifique : UMR7104, Institut National de la Santé et de la Recherche Médicale : U1258,

---

High risk Human papillomaviruses (HPV) are responsible for all cervical, and most anal, vaginal, vulval, penis and back of the throat cancers, for a total of 5% of all human cancers. Proliferation and viral replication are maintained by two oncoproteins, E6 and E7. E6 proteins in particular, have a C-terminal motif called PDZ binding motif (PBM) recognized by PDZ domains, thus disturbing the natural PDZ-PBM human interactome. Based on our database of 65 000 quantitative PDZ-PBM interaction affinities, we are trying to understand the pertubation induced by the viral PBM on the interactome and predict unmeasured affinities.

The measure of affinities gives us an understanding of which PDZ containing proteins are most likely to be perturbed by the viral PBM. However, even if the role of many human proteins is described, having a larger overview of hundreds to thousands of proteins is challenging. To address this problematic, we developed ProFeatMap, an online tool capable of representing any list of proteins as 2D maps showing elements of interests (features) in those proteins. These maps can be completed by RNA expression data across tissues and sorted according to their feature content. Links between some of the proteins can be revealed this way and serve as base for further investigations. This tool is currently used to create a panorama of proteins families with common features. ProFeatMap continues to be developed and refined as a versatile tool for scientists working with proteins.

In parallel, we investigate the possibility to predict currently unmeasured affinities. Our first analysis lead to the usage of k-means clustering methods to reproduce SPOT assay experiments. The SPOT assay shows the affinity of every single mutants of a PBM sequence, offering a set of sequences with all possible amino acids at each position. Preliminary results are promising, capable of recreating predicted SPOT assays highly similar to experimental ones. Based on these results, we plan to predict affinities using machine learning approaches and then adapting this method to affinities in our database. Such a tool would help identify the targets which are most likely to interact, thus helping us focus on these interactions in our experimental measurement planning.

Solubility measurements of the compounds from the French Essential Chemical Library

Claire BOURBAN, Patrick Gizzi, Pascal Villa

UAR 3286 Plateforme de Chimie Biologique Intégrative de Strasbourg (PCBIS) – Centre National de la Recherche Scientifique, Université de Strasbourg
300 boulevard Sébastien Brant CS 10413 67412 Illkirch

Compound solubility can be a major issue in drug discovery. In this context, we present measurements of the kinetic solubility on a subset of the French chemical library. The CNE (Essential Chemical Library) is a selection of 1040 compounds distributed in 96 well plates at a concentration of 5 mM in DMSO.
We developed a full robotic assay in order to obtain a reliable, homogeneous and traceable set of data. The principle is to incubate compounds in physiological buffer during 24 hours followed by a filtration step to separate undissolved compound.
Data will be then used in quantitative structure-property relationship (QSPR) modeling in order to annotate the Complete French chemical Library.

A new machine learning based method for ADRB2 agonist detection using single-ligand dynamic interaction data

Luca Chiesa, Esther Kellenberger

Laboratoire d'Innovation Thérapeutique, Université de Strasbourg, CNRS : UMR7200

The G-protein coupled receptor family is responsible for signaling transduction in many biological processes. The binding of a ligand regulates the signaling by stimulating it (agonist) or inhibiting it (antagonist, inverse agonist). The β2 adrenergic receptor (ADRB2) is one of the most studied GPCR, with many known ligands with an agonistic or antagonistic action.

The ligand binding information provided by crystallographic structures of ADRB2 is often used to improve virtual screening performance, by allowing better separation not only of active and inactive ligands, but also of agonists and antagonists [1][2]. Here, we propose a method that takes into account the conformational dynamics of the ADRB2/ligand reference complex with the aim of improving the biased search towards ligands with specific pharmacological properties.

An ensemble of binding poses was obtained from the crystal structure of ADRB2-agonist complex using molecular dynamics (MD) simulations [3][4]. Key interaction-patterns for agonist activity were selected by a machine learning algorithm. As a test, the developed model was used, in combination with protein-ligand docking, to screen a small library containing well-characterized agonists and antagonists targeting ADRB2.

The proposed technique could be used to post-process docking poses to determine if they can be considered as agonist-like. It can be applied as a filter to remove non relevant poses, non-active ligands, and ligands with an undesired pharmacological effect.

[1] J.Desaphy; E.Raimbaud; P.Ducrot; D.Rognan J. Chem. Inf. Model. 53 (2013) 623-637

[2] A.J.Kooistra; R.Leurs ; I.J.P.De Esch ; C.De Graaf J. Chem. Inf. Model. 55 (2015) 1045-1061.

[3] I.Rodríguez-Espigares;M.Torrens-Fontanals ;J.K.S.Tiemann ;et al *Nat Methods*. 17(2020) 777-787.

[4] R.O.Dror; et al. *Proc. Natl. Acad. Sci. U. S. A.* 108 (2011) 18684– 18689

MiR-30a-3p and miR-30e-3p influence tumor phenotype of head and neck squamous cell carcinoma by targeting TGF-beta/BMP signaling

Ombline Conrad*[1], Mickaël Burgy[1], Sophie Foppolo[2], and Sophie Martin[3]

[1]Laboratoire de bioimagerie et pathologies – Université de Strasbourg, CNRS : UMR7021, Université de Strasbourg, CNRS UMR7021 – France

[2]Laboratoire de Bioimagerie et Pathologies – université de Strasbourg : UMR7021, Centre National de la Recherche Scientifique : UMR7021 – France

[3]Laboratoire de bioimagerie et pathologies, CNRS UMR 7213, Université de Strasbourg, Illkirch – CNRS : UMR7021 – France

Résumé

Head and Neck Squamous Cell Carcinoma (HNSCC) is the 6th most common cancer worldwide (Globocan, 2020). 70% of patients exhibit advanced HNSCC stage at diagnosis and their 5-year survival rate is less than 50% because therapeutic management is only based on the TNM classification and no reliable biomarkers for diagnosis or prognosis are available. In the last few years, miRNAs profile was studied in HNSCC and differences of expression between tumour vs healthy tissue were highlighted. Studies have suggested that miRNAs have the potential to become biomarkers (Sethi et al., 2014). Here, we studied members of the miR-30 family which act as antitumor miRNAs in HNSCC. We investigated the role of miR-30a-3p and miR-30e-3p expression on HNSCC relapses and clinical prognosis. Their expressions were analysed in 122 HNSCC HPV-negative, locally advanced tumours. miRNAs level of expression was correlated with clinical data and it showed that low level of miR-30a-3p and miR-30e-3p was correlated with higher risk of relapses and lower survival rate. Thus, these miRNAs have the potential to become biomarkers for relapses and prognosis. Next, we studied *in vitro* features of relapses and survival by overexpressing miR-30a-3p and miR-30e-p in HNSCC cell lines. Colony formation, proliferation and apoptosis assays showed that survival was reduced when miRNAs were overexpressed in cells. In addition, spheroid evasion assay showed that migration was also reduced compared to control. Then, we wanted to determine the underlying pathway by which miRNAs affected these malignant biological functions. Screening TCGA database and evaluating connection of identified targets using STRING showed that miR-30a-3p and miR-30e-3p target several genes that happen to belong to the TGF-β network. Repression of this pathway was confirmed using RT-qPCR, western blot and immunofluorescence analyses. Pharmacological inhibition of the most affected targets, TGFBR1 and BMPR2, recapitulated effects observed by direct expression of miRNAs, suggesting that miRNAs exert antitumor activity through the inhibition of TGFBR1 and BMPR2. Beside their potential as biomarkers, miRNAs might be used to target one of the most important immunosuppressive pathway in HNSCC.

*Intervenant

Albumin-based biomaterials for the delivery of anti-inflammatory ingredients

Emilie Frisch[1], Eya Aloui[1], Jordan Beurton[1], Claire Medemblik[2], Philippe Lavalle[2], Benoît Frisch[1], Béatrice Heurtault[1]

1 : UMR7199 - Laboratoire de conception et application de molécules bioactives (CAMB) – Biovectorisation, Bioconjugaison et Biomatériaux, Centre National de la Recherche Scientifique, Université de Strasbourg, 74 route du Rhin CS 60024- 67401 Illkirch Cédex – France
2 : UMR1121 - CRBS, Centre de Recherche en Biomédecine de Strasbourg, INSERM/Université de Strasbourg. 1, rue Eugène Boeckel-| 67000 Strasbourg, France

Biomaterials are booming in many therapeutic areas. Whether in the form of implants, prostheses or lenses, these biomaterials are mainly used to replace or regenerate a particular tissue. Despite many advances in terms of material biocompatibility, several complications still need to be overcome. Among these complications, we often observe appearance of a fibrous capsule around the material leading to pain and deformation resulting from the host's immune response[1]. To address these difficulties, we are developing biomaterials with anti-inflammatory properties to decrease the inflammatory reaction.

In this project we are exploiting the advantages of albumin-based biomaterials recently developed within the laboratory (Patent application EP19306387, 2019)[2], in which we aimed to incorporate dexamethasone, a well-known anti-inflammatory glucocorticoid[3]. These albumin-based membranes are obtained by a non-denaturing process in soft conditions of temperature and pressure. They present several interesting physico-chemical properties such as stability in aqueous medium or stability *in vivo* with a constant profile of degradation in time. They can be created with autologous albumin of the chosen target, allowing them to be non-cytotoxic and non-inflammatory. Finally, these membranes are also able to convey numerous active drugs, which we want to exploit.

We first determined the process to incorporate dexamethasone inside the membrane-shaped biomaterials. Then we performed extraction of dexamethasone before quantification through both UV-spectrophotometry and ELISA method. The major part of the dexamethasone embedded in the membranes was extractable and measurable. These first observations led us to study the biomaterials on Raw murine macrophages cell line. No cytotoxicity was observed. Furthermore, after stimulation of the macrophages with lipopolysaccharide (LPS), a pro-inflammatory endotoxin, we showed a major reduction of nitric oxide (NO) and TNF-α productions when dexamethasone was loaded inside materials compared to the non-loaded ones.

In conclusion, formulations of albumin-based membranes loaded with dexamethasone have been developed. The resulting membranes have interesting anti-inflammatory properties. The next steps will be the optimization of the payload and characterization of the dexamethasone release profiles as a function of physicochemical properties of the membranes (depending on methods of preparation, porosity…). *In vivo* study of our biomaterial is also scheduled on inflammatory mouse model such as Crohn disease[4].

1. Sadtler, K., J. Collins, J. D. Byrne, and R. Langer. 2020. 'Parallel evolution of polymer chemistry and immunology: Integrating mechanistic biology with materials design', *Adv Drug Deliv Rev*, 156: 65-79.
2. Aloui, E. De Giorgi, M. Frisch, B. Lavalle, P. Schaaf, P. (2019) ALBUPAD- *Development of biodegradable implantable systems* (EU: Patent application EP19306387)
3. Madamsetty, Vijay Sagar et al. “Dexamethasone: Insights into Pharmacological Aspects, Therapeutic Mechanisms, and Delivery Systems.” ACS biomaterials science & engineering, acsbiomaterials.2c00026. 19 Apr. 2022, doi:10.1021/acsbiomaterials.2c00026
4. Lee A.; De Mei C.; Fereira M.; Marotta R.; Yoon H. Y.; Kim K.; Kwon I. C.; Decuzzi P. Dexamethasone-loaded Polymeric Nanoconstructs for Monitoring and Treating Inflammatory Bowel Disease. Theranostics 2017, 7 (15), 3653–3666. 10.7150/thno.18183.

Development of FRET DNA-PAINT with optimized push-pull fluorene probe

Srijayee Ghosh[1], Guillaume Barnoin[2], Alain Burger[2], Benoît Y. Michel[2], Ludovic Richert[1], Yves Mély[1]

1 : Laboratoire de bioimagerie et pathologies, Université de Strasbourg, CNRS
2 : Institut de Chimie de Nice, Université Côte d'Azur (UCA)

Super-resolution microscopy techniques are useful for bioimaging as they overcome the diffraction limit of light while still being minimally invasive. DNA-PAINT (Points Accumulation for Imaging Nanoscale Topography) is one such technique where the specificity of DNA is exploited by using single-stranded oligonucleotides as docking strands to which fluorescently-labelled complementary (imager) strands bind. FRET (Förster Resonance Energy Transfer) DNA-PAINT is an enhancement of this technique where two complementary strands are labelled with a donor and an acceptor dye respectively, and only the binding of the strands gives acceptor emission. This allows a background-free acquisition and facilitates multicolor imaging.

Our work is based on a new fluorogenic probe DiethylaminoFluoreneKetotriazolyl (DFK) that is promising as donor in FRET DNA-PAINT. It is a nucleobase substitute based on a push-pull fluorene scaffold having very low fluorescence in single strands which limits the background signal from unbound strands and its very large Stokes shift (>200 nm) eliminates cross-excitation with the acceptor (Atto647N dye). There is fluorogenic turn-on of Atto647N emission upon hybridization of DFK-labelled oligonucleotides with complementary Atto647N-labelled strands. Therefore, we demonstrate for the first time the concept of intermolecular DRET (Dark-RET) having a dark donor and a bright acceptor. Its proof of principle has been shown by fluorescence spectroscopy in a cuvette. By using TIRF microscopy, we have validated the concept at the single molecule level where DFK labelled strands were evaluated as imager strands to complementary docking strands labelled with Atto647N immobilized on a coverslip. These single molecules acquisitions showed a perfect co-localization between the fluorescence emitted from excitation of Atto647N alone (by 642 nm laser) and that from excitation of DFK (by 445 nm laser). This shows the proof of concept of the pair DFK/Atto647N as DNA-FRET pair dyes. By modifying the length and concentration of the imager strands labelled with DFK, we could modulate the kinetics of binding events. This gives the possibility to optimize the binding events in order to increase the speed of acquisition of DNA-FRET-PAINT for multiplexed imaging.

Epiregulin (EREG): predictive biomarker of Cetuximab (CTX)-induced ferroptosis in head and neck cancers

Aude Jehl, Sophie Martin

Laboratoire de bioimagerie et pathologies, UMR7021, Université de Strasbourg, CNRS

Head and neck cancers (HNSCC) affect nearly 25.000 people in France each year and cause about 10.000 deaths. Worldwide, they rank sixth among the most frequently observed cancers (*Globocan, 2020*). One of the possible treatments is targeted therapy *via* CTX, a monoclonal antibody targeting the EGFR. Currently, there is no predictive biomarker for the response to CTX that allows optimisation of the patient's therapeutic management.

We recently reported that caveolin-1 (Cav1) expression predicted locoregional relapse of HNSCC due to resistance to CTX and radiotherapy treatment. EREG, an endogenous EGFR ligand, was identified as a key target of Cav1-mediated resistance to treatment (*M. Burgy and **A. Jehl**, Cancers, 2021*). To go further, we studied HNSCC cells in which EREG was depleted using siRNA. Repression of EREG was associated with a decrease of tumour progression features and more specifically the production of ATP reflecting mitochondrial dysfunction. As no apoptosis was observed in EREG-negative cells, we focused on two other types of programmed cell death, namely, autophagy and ferroptosis. According to our results, the expression of autophagy proteins is not affected by the depletion of EREG. Whereas, CTX induces the expression of ULK-1 in EREG-negative cells in a lesser extent than in controls. The fact that control cells engage autophagy more markedly than EREG-negative cells might partially explain why mitochondrial dysfunction is less important in control- than in EREG-negative cells. Turning to ferroptosis, we showed that this latter is decreased in EREG-negative cells. While CTX treatment induced greater ferroptosis in these cells, which is associated with a significant reduction in cell survival as well as ATP production. Therefore, we can suggest that EREG could be used as a predictive biomarker to predict sensitivity to CTX in head and neck cancers. Indeed, tumours with low expression of EREG may respond better to this targeted therapy.

Moreover, the data also suggest that patients with tumours expressing low levels of EREG might be treated with combination of CTX and a nutrient transporter inhibitor, thereby disrupting redox homeostasis resulting in the accumulation of peroxidized lipids and a deficit of antioxidant molecules. This disruption will promote ferroptosis for cytolytic purposes.

An automated method to determine the interaction of transmembrane domains

Thomas Kuntzel[1], Lucas Pham-Van[1], Sophie Gioria[2], Pascal Villa[2], Merveille Eguida[3], Didier Rognan[3], Dominique Bagnard[1]

1 : Biotechnologie et signalisation cellulaire, UMR7242, Université de Strasbourg - Centre national de la recherche scientifique
2 : Plate-forme de chimie biologique intégrative de Strasbourg, Université de Strasbourg - Centre National de la Recherche Scientifique : UAR3286 / UMS3286
3 : Laboratoire d'innovation thérapeutique, UMR7200, Université de Strasbourg - Centre national de la recherche scientifique, Institut de Chimie du CNRS

Membrane receptors oligomerize or interact with other co-receptors in order to get activated or regulated. These interactions happen in both extra- and intracellular domains. However, even if the transmembrane domain (TMD) is sometimes seen as having a role only for the anchoring in the membrane, increasing evidence is accumulating that it also has a crucial role for the interaction of receptor subunits or co-receptors. Targeting this specific domain represents an interesting approach for the development of substances impeding these interactions.

In order to systematically study the potential role of the TMD in the activation of membrane receptors, we developed a screening method relying on an *in silico* modelling approach followed by an automated biological validation *in vitro*, based on a BRET assay assisted by automatic pipetting robots. Bioluminescence Resonance Energy Transfer is an approach where a donor (Rluc) is transferring its bioluminescence energy to an acceptor (eYFP) in presence of the luciferase substrate (Coelenterazine) if they are in close proximity. In this case, a fluorescent signal can be measured. These reporters are coupled to the proteins for which an interaction is measured and these constructs are coded in plasmids, transfected in HEK-293 cells.

Before being able to perform this screening, we needed to develop and validate the protocol using the pipetting robots. To this end, we relied on a 4x4 matrix performed by hand. We validated each step of the protocol, beginning with the mix of plasmids followed by transfection, washes, deposit of the substrate and readings. Each step has also been extensively controlled for repeatability in order to follow the principles of quality management.

First, we focused on tyrosine kinase receptors (RTKs) and we were able to determine biologically the interaction of all TMD for the 58 identified human RTKs, corresponding to 3364 interactions. This first screening permitted to generate precious data for the RTKs receptor family widely used as a drug target. Actual treatments targeting RTKs present various success rates due to resistance mechanisms such as the redundancy of signalling cascades. The information given by our screening opens the possibility to develop active compounds with multiple targets and thereby inhibiting redundant signalling pathways.

Effect of the APP gene humanization in rat models of Down Syndrome

Pellen M[1], Maréchal D[1], Pani G[1], Birling MC[2], Lindner L[2], Cayrou P[2], Prevost G[2], Jacquot S[2], Pereira De Vasconcelos A[3], Cassel JC[3], Hérault Y[1,2]

[1]Equipe Physiopathologie de la trisomie 21 et des maladies rares à effet de dose causant des déficiences intellectuelles ou des troubles du spectre autistique et autres comorbidités - Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC) - UMR 7104 CNRS - U 1258 INSERM - Université de Strasbourg - France

[2]Institut Clinique de la Souris (ICS) - France

[3]Equipe Epigénétique et Dynamique des Systèmes de Mémoire - Laboratoire de Neurosciences Cognitives et Adaptatives (LNCA) - UMR 7364 CNRS - Université de Strasbourg - France

Down Syndrome (DS) is the first cause of intellectual disability with a genetic origin. It is due to an extra copy of the chromosome 21 (Hsa21) and affects 1 out of 800 newborns. People with DS show specific phenotypes together with several comorbidities including Alzheimer's disease (AD). Most people with DS develop amyloid plaques, one of the characteristics of AD, by the age of 40 and around 68-80% of them will develop AD. This is due to the presence in 3 copies of the *APP* gene which is located on the Hsa21. This gene is known for its involvement in AD and its presence in 3 copies is sufficient to induce the development of an Alzheimer's type dementia.

In the rat, the homologous region of the Hsa21 is found on two different chromosomes: chromosomes 11 and 20 (noted Rno11/20). We have created rat models carrying the duplication of one or both regions to better understand the role of each of them in the development of DS. These models were characterized at the behavioral level. We found that most of the behavioral deficits were linked to the duplication of the Rno11 region where the *App* gene is localized. However, rats do not develop amyloid plaques. Thus, even if rats with a duplication of the Rno11 region have 3 copies of the *App* gene we cannot study the development of AD in this model. This is due to 3 amino acid changes in the APP sequence between rodent and human. Thus, we changed these 3 amino acids in the rat sequence to have them identical to the human sequence. So, we created a rat model humanized for the *App* gene. This humanized version of *App* was then introduced in our rat models of DS to be able to fully model AD in the context of DS. Here, we present the impact of these *App* humanization at the behavioral level.

Structure and function of the C9ORF72-SMCR8-WDR41 complex and its implication in Amyotrophic lateral Sclerosis (ALS)

David Pietri*[1], Manon Boivin[1], Véronique Pfister[1], and Nicolas Charlet-Berguerand[1]

[1]Institut de Génétique et de Biologie Moléculaire et Cellulaire – université de Strasbourg : UMR7104, Institut National de la Santé et de la Recherche Médicale : U1258, Centre National de la Recherche Scientifique : UMR7104 – France

Résumé

Amyotrophic lateral sclerosis (ALS), the 3rd most common neurodegenerative disease, is characterized by degeneration of both upper and spinal motor neurons, resulting in skeletal muscle paralysis and death from respiratory failure generally in 3 to 5 years. The main genetic cause of ALS is an expansion of GGGGCC repeats in the C9ORF72 gene. These repeats promote DNA epigenetic changes that silence C9ORF72 expression. However, very little is known on the molecular and cellular roles of this protein.
Recently we found that C9ORF72 is part of a large complex (Sellier et al., 2016; Boivin et al., 2020) and to explore its functions, I solved its 3D structure by cryo-EM. While we successfully produce, purify and image C9ORF72 complex, we noted that a large part was not resolved due to conformational flexibility, most likely as other proteins are require to stabilize it. Thus, I searched for novel interactants of C9ORF72 and through extensive immunoprecipitation and mass spectrometry analyzes, we found a novel interaction between C9ORF72 and ARL14, a small GTPase of ill-defined function. Furthermore, I observed by super-resolution microscopy that the C9ORF72 protein re-localizes to lysosomes upon mTOR and autophagy activation. Importantly, I also found a role of C9ORF72 in Autophagic Lysosome Reformation (ALR), a novel mechanism explaining regeneration and biogenesis of new lysosomes after mTOR and autophagy activation.

Overall, my PHD work unveils a novel partner (ARL14) to the C9ORF72 complex and an exciting novel function for this complex in lysosome biogenesis. This is important as dysfunctions of lysosomes may explain neuronal cell death in ALS. It would be essential to finalize this work and determine the structure-functions of the C9ORF72 complex and its implication in ALS. This is highly relevant as elucidating the molecular mechanisms underlying ALS will open novel therapeutic route for this devastating disease.

Boivin, M et al, 2020. Reduced autophagy upon C9ORF72 loss synergizes with dipeptide repeat protein toxicity in G4C2 repeat expansion disorders. EMBO J 39, e100574

Sellier, C et al, 2016. Loss of C9ORF72 impairs autophagy and synergizes with polyQ Ataxin-2 to induce motor neuron dysfunction and cell death. EMBO J 35, 1276–1297

*Intervenant

Comprehensive analysis of commercial fragment libraries

Julia Revillo Imbernon[1], Célien Jacquemard[1], Guillaume Bret[1], Gilles Marcou[2], Esther Kellenberger[1]

1 : Laboratoire d'Innovation Thérapeutique, UMR7200, Centre National de la Recherche Scientifique, Université de Strasbourg, Institut de Chimie du CNRS
2 : Laboratoire de Chémoinformatique, Chimie de la matière complexe

---

For the last 25 years, Fragment-Based Drug Discovery (FBDD) has widely increased in popularity and proven its interest by connecting many worlds.[1] It has become an alternative to High-Throughput Screening (HTS) and has the advantage of covering a large chemical space with a small number of fragments while providing structural information for the elaboration of hit into druglike compound.[2]

This work aims to analyze the composition of commercial fragment libraries. We focused on important topics on FBDD: molecular obesity[3], three-dimensionality[4] and chemical diversity.

We collected the fragments of 86 freely-available libraries from 14 suppliers. We determined the chemical descriptors related to the Rule of 3[5], and three-dimensional descriptors (PBF, 3D-PSA...). for the full ensemble of fragments. To assess the chemical diversity of libraries, we studied the number and frequencies of chemical scaffolds, and analyzed the fragment space using Generative Topographic Map[6] (GTM).

We studied 754 646 molecules, 512 284 after filtering the duplicates. The small libraries, containing a maximum of 2000 molecules, are the most interesting with respect to experimental testing. The analysis of the 2D and 3D descriptors showed that MW and logP distributions are globally well balanced in small libraries and that there is a bias towards flat molecules. The scaffold analysis revealed a sur-representation of very simple scaffolds as well as many scaffolds present in only one molecule. Finally, the analysis of the GTM landscapes allowed the systematic comparison of the libraries by pairs. It also allowed to evaluate whether a library is representative of the full fragments set.

In conclusion, our results provide guidelines for the selection or the design of an adequate library for a specific project.

[1] Romasanta A. K. S., van der Sijde P., Hellsten I., Hubbard R. E., Keseru G. M., van Muijlwijk-Koezen J., de Esch I. J. P. *Drug Discovery Today* **23** 1596-1609 (2018)

[2] Lipinski C., Hopkins A. *Nature* **432** 855-861 (2004)

[3] Hann M. M., Keserü G. *Nat Rev Drug Discov* **11** 355–365 (2012)

[4] Lovering F., Bikker J. Humblet C. *J. Med. Chem.* **52** 6752-6756 (2009)

[5] Congreve M., Carr, R., Murray, C. & Jhoti, H. *Drug Discov. Today* **8** 876– 877 (2003)

[6] Kireeva N., Baskin I., Gaspar H. A., Horvath D., Marcou G., Varnek A. *Mol. Inf.* **31** 301-312 (2012)

Fluoroproline scan enables to visualize conformational ensemble in proline-rich peptide sequence

Abstract :

Proline is unique between the 20 natural amino acids - it is the only imino acid and its distinctive cyclic structure rends the backbone structure more rigid thus it is more susceptible to undergo cis-trans isomerisation. Moreover, it is the residue which is the most found in intrinsically disordered proteins (IDPs) (1) which are able to engage in biological activities and perform impossible tricks that are highly unlikely for ordered proteins (2).

This study focuses on the isomerisation of prolines in an IDP rich in this residue. In fact, this Xaa-Pro bond conformation is important for biological functions : it affects the binding and the folding of a protein. On the example of Dynamine 2 fragment, sequentially all the prolines were replaced by a 4R-fluoroproline which enhances the trans isomer or a 4S-fluoroproline which enhances the cis (3) . The effect of fluoroproline incorporation in the peptide was observed by ^{19}F NMR to dress the conformational ensemble of the wild type peptide.

(1) Theillet, F.-X. *et al.* The alphabet of intrinsic disorder: I. Act like a Pro: On the abundance and roles of proline residues in intrinsically disordered proteins. *Intrinsically Disord Proteins* **1**, e24360 (2013).

(2) Uversky, V. N. Intrinsically Disordered Proteins and Their “Mysterious” (Meta)Physics. *Frontiers in Physics* **7**, (2019).

(3) Sinnaeve, D. *et al.* Fluorine NMR study of proline-rich sequences using fluoroprolines. *Magnetic Resonance* **2**, 795 (2021).

Identification of an N-acylated-DArg-Leu-NH2 Dipeptide as a Highly Selective Neuropeptide FF1 Receptor Antagonist That Potently Prevents Opioid-Induced Hyperalgesia

¶

Raphaëlle Quillet[1], Séverine Schneider[2], Valérie Utard[1], Armand Drieu la Rochelle[1], Khadija Elhabazi[1], Jo Beldring Henningsen[3], Patrick Gizzi[4], Martine Schmitt[2], Valérie Kugler[1], Valérie Simonneaux[3], Brigitte Ilien[1], Frédéric Simonin[1]* and Frédéric Bihel[2]*

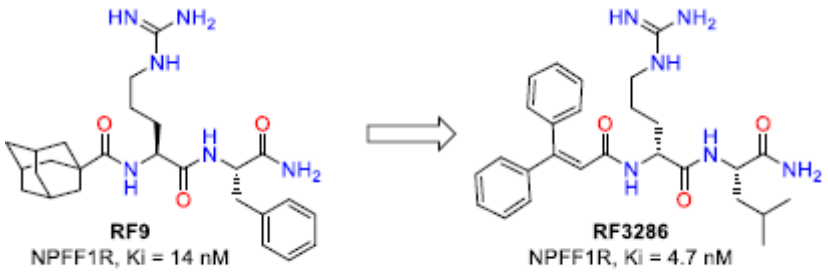
¶

1 Biotechnologie et Signalisation Cellulaire, UMR 7242 CNRS, Université de Strasbourg, 67412 Illkirch, France ; 2 Laboratoire d'InnovationThérapeutique, UMR 7200 CNRS, Université de Strasbourg, 67401 Illkirch, France ; 3 Institut des Neurosciences Cellulaires et Intégratives, UPR 3212 CNRS, 67000 Strasbourg, France ; 4 TechMedIll, UMS 3286 CNRS, Université de Strasbourg, 67412 Illkirch, France

¶

¶

RFamide-related peptide-3 (RFRP-3) and neuropeptide FF (NPFF) target two different receptor subtypes called neuropeptide FF1 (NPFF1R) and neuropeptide FF2 (NPFF2R) that modulate several functions. However, the study of their respective role is severely limited by the absence of selective blockers. We describe here the design of a highly selective NPFF1R antagonist called RF3286, which potently blocks RFRP-3-induced hyperalgesia in mice and luteinizing hormone release in hamsters. We then showed that the pharmacological blockade of NPFF1R in mice prevents the development of fentanyl-induced hyperalgesia while preserving its analgesic effect. Altogether, our data indicate that RF3286 represents a useful pharmacological tool to study the involvement of the NPFF1R/RFRP-3 system in different functions and different species. Thanks to this compound, we showed that this system is critically involved in the development of opioid-induced hyperalgesia, suggesting that NPFF1R antagonists might represent promising therapeutic tools to improve the use of opioids in the treatment of chronic pain.



Reference: Quillet et al. *J Med. Chem.* **2021,** 64, *11*, 7555–7564
https://doi.org/10.1021/acs.jmedchem.1c00256

Development of an antitumor vaccine approach based on the delivery of messenger RNA

Mohammed-Karim SEBANE [1,2], Candice DUSSOUILLEZ [1], Sylvie FOURNEL [1], Antoine KICHLER [1]

1: Team 3BIO (Biovectorisation, Bioconjugaison and Biomaterials), UMR7199 Design and Application of Bioactive Molecules, University of Strasbourg, 74 Rte du Rhin, 67400 Illkirch-Graffenstaden

2: Doctoral School of Life and Health Sciences, University of Strasbourg, 46 Bd de la Victoire, 67000 Strasbourg

---

Cancer immunotherapy is defined as the ability to mobilize the host's immune system to kill cancer cells. It has recently taken a central role within mainstream oncology with the use of immune checkpoint inhibitors and has shown unprecedented clinical responses in patients. Despite this success, broad immunotherapy can result in severe adverse effects such as autoimmunity, highlighting the need for new therapies. In the last decades, therapeutic cancer vaccines have proven to be able to induce strong immune responses with little-to-non adverse effects [1]. Capable of eliciting exceptionally strong immune responses, RNA has emerged as an attractive vaccine platform for cancer therapy [2]. Thus, we propose to develop innovative mRNA-based vaccine formulations that will allow the establishment of an effective anti-tumor immune response.

Currently, endosomal entrapment of mRNA-based cargos remains a critical unsolved barrier for efficient delivery, translation, and activation of the immune system by the mRNA [3]. As a part of our work, we designed pH-triggered cell penetrating peptides (CPPs) derived from viral fusogenic peptides [4]. We investigate the ability of those CPPs to efficiently transfect mRNA into dendritic cells (DCs), the antigen presenting cells (APCs) which are the initiators of the immune response. By using mRNA coding for the reporter genes luciferase and green fluorescent protein, we observe a strong transfection efficiency of various APCs (murine macrophages RAW264.7 and mouse DC2.4) as compared to commercial transfection agent Lipofectamine. As RNA is also a danger signal to the immune system, we confirm the ability of our constructs to induce the activation of APCs by analyzing the overexpression of activation markers (CD40, CD86) on the surface of those cells by flow cytometry.

Henceforward, we aim to ensure the ability of our formulations to induce the presentation of the mRNA encoded antigen by the DCs and their ability to induce the activation of effector immune cells by co-culture with T-cells (responsible for the killing of cancer cells). Our molecules could thus represent and easy-to-formulate platform for mRNA vaccination that could be very interesting for cancer therapy.

1. Heuts, J., Jiskoot, W., Ossendorp, F. & van der Maaden, K. Cationic Nanoparticle-Based Cancer Vaccines. *Pharmaceutics* **13** (2021).
2. Tahtinen, S. *et al.* IL-1 and IL-1ra are key regulators of the inflammatory response to RNA vaccines. *Nature immunology* **23** (2022).
3. Kranz, L. *et al.* Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. *Nature* **534** (2016).
4. Plank, C., Oberhauser, B., Mechtler, K., Koch, C. & Wagner, E. The influence of endosome-disruptive peptides on gene transfer using synthetic virus-like gene transfer systems. *The Journal of biological chemistry* **269** (1994).

Photophysical characterization of a fluorescent analogue of guanine, as a new tool for characterizing the structure and dynamics of nucleic acids

Olha Tkach,[1] Nicolas Humbert,[1] Pascal Didier,[1] Ludovic Richert,[1] Dmytro Dziuba,[1] Yitzhak Tor,[2] Lara Martinez-Fernandez,[3] Roberto Improta,[4] Yves Mély [1]

1 : UMR 7021 Laboratoire de Bioimagerie et Pathologies (LBP), Universite´ de Strasbourg, 74 route du Rhin, 67401 Illkirch, France.
2 : Department of Chemistry and Biochemistry University of California, San Diego, 9500 Gilman Dr, La Jolla, CA 92093-0358, USA
3 : Departamento de Química, Facultad de Ciencias and Institute for Advanced Research in Chemistry (IADCHEM), Universidad Autónoma de Madrid, Campus de Excelencia UAM-CSIC, 28049 Madrid, Spain
4 : Consiglio Nazionale delle Ricerche Istituto di Biostrutture e Bioimmagini Via Mezzocannone 16, 80134 Napoli, Italy.

The study of dynamic interactions of nucleic acids with proteins via fluorescence techniques is of high interest. Consequently, development of environmental sensitive fluorescent nucleoside analogues for site-selectively characterization of the structure and dynamics of nucleic acids is of high demand 1. Isothiazolo-guanine (^{tz}G) 2, an isomorphic and isofunctional fluorescent analogue of guanosine (G), is of particular interest in this respect. ^{tz}G shows H-bonding and tautomeric preferences similar to G and can participate in H-bonding with its Hoogsteen face. To further exploit the potential of this probe and properly interpret its spectroscopic changes, it is critical to fully understand its photophysics.

In this work we characterized the steady-state and time-resolved fluorescence properties of free ^{tz}G in different solvents. Our data revealed the existence of two ground-state tautomers of ^{tz}G in protic solvents that differ by their absorption and emission spectra, suggested by quantum mechanical (QM) calculations to be the H1 and H3 keto-amino species. The H3 tautomer is stabilized in protic solvents, but is absent in aprotic media. QM calculations rationalize their non radiative decays and explain the large differences in quantum yield between the two tautomers. Time-resolved fluorescence measurements further evidenced the existence of excited-state reactions (ESR) both in protic and aprotic solvents. Altogether the experimental data and QM calculations allowed us to identify the species involved in the ESR and propose a model for describing the fundamental photophysics of ^{tz}G in both protic and aprotic solvents.

Ultimately, ^{tz}G will be included in oligonucleotides and spectroscopically characterized to assess the impact of the nucleic acid context on ^{tz}G properties. The unique properties of ^{tz}G are of particular interest for site-specific characterization of molecular interactions between proteins and nucleic acids.

1. Dziuba, D. et al. Fundamental Photophysics of Isomorphic and Expanded Fluorescent Nucleoside Analogues. *Chem. Soc. Rev.* 50 (12), 7062–7107 (2021).
2. Rovira, A. R. et al. Chemical Mutagenesis of an Emissive RNA Alphabet. *J. Am. Chem. Soc.* 137 (46), 14602–14605 (2015).

Development and validation of miniaturized spheroid assays

Christel Valencia*[1], Adeline Obrecht[2], and Pascal Villa[1]

[1]Plate-forme de chimie biologique intégrative de Strasbourg – université de Strasbourg, CNRS : UMS3286 – France

[2]Plate-forme de chimie biologique intégrative de Strasbourg – université de Strasbourg, CNRS : UMS3286 – France

Résumé

3D cellular models, such as spheroids, offer an intermediate level of complexity between single-layer cell culture and the use of animal models, reproducing in vitro organization of a micro-tumor. These models have been shown to be more predictive of treatment response than single-layer cell culture models. Here we describe the development and validation of miniaturized (96-well) spheroid assays for quantification of spheroid growth and evaluation of cytotoxic effects of compounds.

*Intervenant

Fluorescent Turn-ON Detection of Bacteria with Targeted Bioconjugates

Lucille Weiss,[a] Patrick Wagner,[a] Stéphanie Riché,[a] Dmytro Dziuba,[b] Patrice Rassam,[b] Yves Mély,[b] Pierre Fechter,[c] Dominique Bonnet,[a*] Julie Karpenko.[a*]

a. Laboratoire d'Innovation Thérapeutique, UMR 7200 CNRS/Université de Strasbourg, Institut du Médicament de Strasbourg, 74 route du Rhin, 67401 Illkirch.[2]

b. Laboratoire de Bioimagerie et Pathologies, UMR 7021 CNRS/Université de Strasbourg, 74 route du Rhin, 67401 Illkirch.

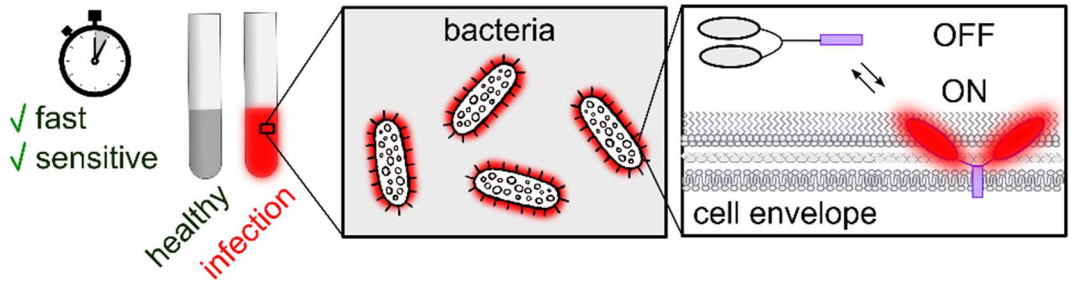
c. Biotechnologie et Signalisation Cellulaire, UMR 7272 CRNS/Université de Strasbourg, ESBS, 300 Bd Sébastien Brant, 67412 Illkirch.

Résumé:

Rapid detection and identification of bacterial infections are among the key actions to prevent antibiotic misuse and limit the spread of resistant bacteria. An instantaneous mix-and-read assay for the detection of bacteria in human body fluids and identification of its antibiotic susceptibility would be of high value to public health and society. Fluorescent probes hold great promise in the field of biomedical express diagnostics.[1] However, existing fluorescent bacterial probes are poorly suitable for direct and fast detection of bacterial infections in complex biological samples, such as blood or urine.[2]

Here we propose a new concept of targeted fluorescent turn-on probes for bacteria, based on aggregation caused quenching (ACQ).[3,4] The probes are composed of bacteria-targeting vectors (antibiotics, antimicrobial peptides) and covalent dimers of far-red aromatic dyes, which exist in water in the form of non-fluorescent π- stacked H-aggregates (the OFF state). In a less polar medium (such as organic solvents or components of the bacterial cell envelope), the H-aggregate is disturbed, and the fluorescence of the probes is restored (the ON state).

A set of Cy5.5 and squaraine dimers have been synthesized and coupled to bacteria-targeting molecular vectors. Fluorescence studies in a series of solvents demonstrated the ability of these probes to generate a strong fluorescence turn-on response when passing from an aqueous to a less polar medium. The most efficient probes were characterized by high selectivity for bacterial *vs* eukaryotic cells and enabled the detection of living bacteria in no-wash conditions by fluorescence spectroscopy and fluorescence microscopy.



1. Kobayashi, H.; Ogawa, M.; Alford, R.; Choyke, P. L.; Urano, Y. *Chem. Rev.* **2010**, *110*, 2620. DOI: 10.1021/cr900263j
2. Mills,B.; Bradley, M.; Dhaliwal,K. Clin Transl Imaging, 2016, 4, 163. DOI: 10.1007/s40336-016-0180-0
3. Karpenko, I; Collot, M.; Richert, L.; Valencia, C.; Villa, P.; Mély, Y.; Hibert, M.; Bonnet, D.; Klymchenko, A. JACS, 2015, 137 (1), 405–412. DOI: 10.1021/ja5111267
4. Esteoulle, L.; Daubeuf, F.; Collot, M; Riché, S.; Durroux, T.; Brasse, D.; Marchand, P.; Karpenko, J.; Klymchenko, A.; Bonnet, D. *Chem. Sci.*, **2020**, *11*, 6824-6829. DOI: 10.1039/d0sc01018a

Keywords: fluorescent probes; bacterial detection; turn-on fluorophores

Targeting the GASP motif: Characterization of polyclonal anti-GPRASP1 antibodies that inhibit protein-protein interactions

Gabrielle Zeder-Lutz[1], Olivier Bornert[1], Rosine Fellmann-Clauss[1], Adeline Obrecht[2], Thibaud Tranchant[1], Sarah Bouteben[3], Juliette Kaeffer[1], Raphaëlle Quillet[1], Pascal Villa[2], Renaud Wagner[1,3], Sandra Lecat[1] and Frédéric Simonin[1]

1: Biotechnology and cell signaling, GPCRs in Pain and Inflammation, UMR7242 CNRS, University of Strasbourg, 300 bd Sébastien Brant BP 10413 F-67412 Illkirch, ITI Institut du Médicament de Strasbourg

2: Plateforme de chimie biologique integrative de Strasbourg, UAR3286*, CNRS, University of Strasbourg, 300 bd Sébastien Brant BP 10413 F-67412 Illkirch, ITI Institut du Médicament de Strasbourg.*

3: Biotechnology and cell signaling, IMPReSs facility for Integral Membrane Proteins Research and Services UMR7242 CNRS, University of Strasbourg, 300 bd Sébastien Brant BP 10413 F-67412 Illkirch

---

The GASP motifs consist of 20 amino acid repeats unique to the GPRASP/ARMCX (GPCR-associated sorting protein) / (ARMadillo repeat-Containing proteins on the X chromosome) proteins subfamily one. Previous results have suggested an implication of the GASP motifs in mediating protein-protein interactions in particular with G protein-coupled receptors [1, 2]. The purpose of this study was to identify and characterize anti-GASP motifs antibodies and to investigate their potential inhibitory functions.

An in-house rabbit polyclonal serum directed against GPRASP1 protein contains anti-GASP motif antibodies indicating that GASP motifs are immunogenic. The affinity purified anti-GASP motif antibodies can detect GPRASP1 and GPRASP2 proteins in Western blot, immunoprecipitation and immunofluorescence experiments. A mutant of GPRASP2 in which the most conserved hydrophobic core of the GASP motifs are mutated is no more detectable by the subpopulation of anti-GASP motif antibodies. The paralogue proteins GPRASP3 and ARMCX5 that contain less and most divergent GASP motifs are not detected by the subpopulation of anti-GASP motif antibodies. The characterization of the affinity purified anti-GASP motif antibodies by ELISA and Surface Plasmon Resonance assays suggest that GASP motifs function as multivalent epitopes. We have set-up a miniaturized Amplified Luminescent Proximity Homogeneous AlphaScreen® assay based on the interaction between purified beta2-adrenergic receptor and the central domain of GPRASP1 containing 19 GASP motifs. In this protein-protein interaction assay, anti-GASP motif antibodies have inhibitory properties.

The use of characterized GASP motif antibodies further confirms the function of GASP motifs as mediators of protein-protein interactions.

1. Bornert O. *et al*., Identification of a novel protein-protein interaction motif mediating interaction of GPCR-associated sorting proteins with G protein-coupled receptors. *PLoS One*. **8**, 1-11 (2013).

2. Kaeffer J. *et al*., GPRASP/ARMCX protein family: potential involvement in health and diseases revealed by their novel interacting partners. Curr Top Med Chem. **21**, 227-254 (2021).

Involvement of Retinoic Acid Receptorβ Signaling in the Physiopathology of Huntington Disease

Nicolas Zinter[1234*]; Valentin Freixial[1234]; Damien Plassard[1234]; Tao Ye[1234]; Valérie Fraulob[1234]; Wojciech Krezel[1234*]

1-Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), 67404, Illkirch, France
2 Inserm, U 1258, 67404 Illkirch, France
3 CNRS UMR 7104, 67404 Illkirch, France
4 Université de Strasbourg, 67, France
** Corresponding Authors (zintern@igbmc.fr ; krezel@igbmc.fr)*

Huntington Disease (HD) is a neurodegenerative pathology characterized by severe motor impairment and progressive cognitive disabilities. HD is an autosomal dominant basal ganglia disease caused by a mutation in huntingtin (Htt) coding gene. This mutation corresponds to the abnormally long (more than 27) CAG trinucleotide repeat expansion, leading to the production of a toxic mutant protein (mHtt) that aggregates in neuronal cells. The toxicity of mHtt is inducing the loss of medium spiny neurons expressing dopamine 2 receptor (striatopallidal pathway), driving to the loss of function of striatum. One of the most studied HD mouse model is the R6.1 model. These mice have a six-months lifespan and are reproducing many HD-like symptoms such as axons hypo myelinization or as increased adult neurogenesis in the subventricular zone of striatum, that is disturbing cell cycle signaling.

Transcriptomic analyses of striatal samples from HD patients and mouse models of this diseas show a 50% loss of expression of Retinoic Acid Receptor β (RARβ). Such reduction of RARβ expression is potentially due to its partial sequestration by mHtt aggregates. We hypothesized that the decreased expression of RARβ in HD condition contributes to its pathophysiology.

To investigate the role of the RARβ in the HD pathology, we used an experimental approach to evaluate whether mHtt in R6.1 mouse model of HD (R6.1 Tg/Ø) can synergize with RARβ haploinsufficiency (RARβ+/-) to accelerate HD-like phenotypes in a double mutant mice (R6.1 Tg/Ø ; RARβ+/-).

We found that R6.1 Tg/Ø ; RARβ+/- displayed motor coordination deficit in the rotarod assay and hyperactivity in actimetric cages which were stronger than in compound mutant mice at 2 months of age. Moreover, transcriptomic analyses of striatum samples revealed more synergistic changes related to cell cycle and myelination in the double mutant mice. Such data support globally pathogenic contribution of compromised RARβ signaling in the pathophysiology of HD.

Authors wanted to address many thanks to ANR RAinRARE for their financial support, to Alexis Simon and Peggy Mellul for animal care and to Dr. Kenny Schumacher for his help in sequencing graphical representations.