



Title of the PhD project:

Novel KCC2 enhancers for epilepsy and related disorders: Molecular modeling and virtual screening for drug discovery

PhD Supervisor

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Address : 17 rue du Fer a Moulin, 75005 Paris

Laboratory : Institut du Fer a Moulin, UMR-S 1270

Title of the team : Plasticity in Cortical Networks & Epilepsy

Team leader (if different) :

Doctoral School : ED3C

Overview of the scientific projects of the team

Our team explores the properties of cortical synapses and networks, the mechanisms governing their plasticity and their alteration in the pathology. Our work focuses in particular on GABAergic synapses and networks underlying inhibitory neurotransmission in the adult brain. Our aim is to understand the molecular mechanisms that regulate the organization, the function and the plasticity of GABAergic synapses and networks.

Our work combines advanced imaging techniques (SPT, PALM/STORM) with *in vitro* and *in vivo* electrophysiology in animal models and human brain tissue. We currently collaborate with geneticists and clinicians in the field of epilepsy as well as experts in super-resolution imaging and modelling of neurons and networks.

Main publications since January 1^{er}, 2016

Otsu Y, Donneger F, Schwartz EJ, **Poncer JC** (2020) Chloride transport and the dynamics of GABA signaling in hippocampal parvalbumin-expressing interneurons. ***Journal of Physiology (London)*** 598, 1865-1880

Goutierre M, Al Awabdh S, Donneger F, François E, Gomez-Dominguez D, Irinopoulou I, de la Prida L, **Poncer JC** (2019) KCC2 regulates neuronal excitability and hippocampal activity via interaction with Task-3 channels. ***Cell Reports*** 28: 91-10 [Comment in *Epilepsy Curr.* 2019]

Heubl M, Zang J, Pressey JC, Al Awabdh S, Renner M, Gomez-Castro F, Moutkine I, Eugène E, Russeau M, Kahle KT, **Poncer JC**, Lévi S (2017) GABAA receptor-dependent synaptic inhibition rapidly tunes KCC2 activity via the Cl⁻-sensitive WNK1 kinase. ***Nature Communications*** 8: 1776

Belvindrah R, Natarajan K, Shabajee P, Bruel-Jungerman E, Bernard J, Goutierre M, Moutkine I, Jaglin X, Savariradjane M, Irinopoulou T, **Poncer JC**, Keays D, Janke C, Francis F (2017) Mutation of Tuba1a leads to straighter microtubules, perturbing saltatory neuronal migration. *J. Cell Biology* 216:2443-2461

Giralt A, Brito V, Chevy Q, Simonnet C, Otsu Y, Cifuentes-Díaz C, de Pins B, Coura R, Alberch J, Ginés S, **Poncer JC**, Girault JA (2017) Pyk2 modulates hippocampal excitatory synapses and mediates Huntington's disease cognitive deficits. *Nature Communications* 8:15592

PhD Co-Supervisor

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Laboratory : UMR8601-CNRS

Title of the team : Métabolisme, Pharmacochimie et Neurochimie

Team leader (if different) : Marie-Agnes Sari

Doctoral School : MTCl

Overview of the scientific projects of the team

Groupe thématique (Medicinal Chemistry for the Modulation of Synaptic Transmission) au sein de l'équipe "Biological Chemistry":

The group is focused on small molecules that target receptors and transporters of synaptic transmission and their mechanisms. Using synthetic organic chemistry and molecular modeling, we develop pharmacological and therapeutic tools and investigate receptor activation and neurotransmitter transport. The team is involved in various projects from the initial design (molecules, 3D models...) all along to the *in vivo* studies. The interdisciplinary projects are made possible through numerous collaborations in the fields of pharmacology, molecular biology, cellular biology and behavior. Thus, molecules that are developed in the group allow investigations of animal models of Parkinson's disease motor symptoms, psychiatric diseases, epilepsy, cognition, pain and addiction as well as imaging and activation or transport mechanism elucidation.

Main publications since January 1^{er}, 2016

Liu H, Yi P, Zhao W, Wu Y, **Acher F**, Pin JP, Liu J, Rondard P. Illuminating the allosteric modulation of the calcium-sensing receptor. *Proc Natl Acad Sci U S A* 2020, 117(35), 21711-21722. doi: 10.1073/pnas.1922231117.

Dubois L, Pietrancosta N, Cabaye A, Fanget I, Debacker C, Gilormini PA, Dansette PM, Dairou J, Biot C, Froissart R, Goupil-Lamy A, Bertrand HO, **Acher FC**, McCort-Tranchepain I, Gasnier B, Anne C. Amino Acids Bearing Aromatic or Heteroaromatic Substituents as a New

Class of Ligands for the Lysosomal Sialic Acid Transporter Sialin. **J Med Chem.** 2020, 63(15):8231-8249.

Orrico-Sanchez A., Chausset-Boissarie L., Alves de Sousa R., Coutens B., Rezai Amin S., Vialou V., Louis F., Hessani A., Dansette P.M., Zornoza T., Gruszczynski C., Giros B., Guiard B.P., **Acher F.**, Pietrancosta N., Gautron S. Antidepressant efficacy of a selective organic cation transporter blocker in a mouse model of depression. **Mol Psychiatry.** 2020, 25(6) 1245-1259.

Habrian C., Levitz J., Vyklicky V., Fu Z., Hoagland A., McCort-Tranchepain I., **Acher F.** and Isacoff E. Y. Novel conformational pathway provides unique sensitivity and dynamics to a synaptic mGluR. **Nature Commun** 2019, 10 (1), 5572

Selvam, C., Lemasson, I. A., Brabet, I., Oueslati, N., Karaman, B., Cabaye, A., Tora, A. S., Commare, B., Courtiol, T., Cesarini, S., McCort-Tranchepain, I., Rigault, D., Mony, L., Bessiron, T., McLean, H., Leroux, F. R., Colobert, F., Daniel, H. Goupil-Lamy, A., Bertrand, H.-O., Goudet, C., Pin, J.-P. and **Acher, F. C.** Increased potency and selectivity for group-III metabotropic glutamate receptor agonists binding at dual sites. **J Med Chem** 2018, 61, 1969-1989.

Doctoral Project

Title: Novel KCC2 enhancers for epilepsy and related disorders: Molecular modeling and virtual screening for drug discovery

Abstract :

Altered neuronal chloride regulation is a hallmark of many neurological disorders including epilepsy. Perturbed expression/function of the cation-chloride cotransporters KCC2 and NKCC1 impair GABA signalling and neuronal excitability. Whereas inhibiting NKCC1 function lacks CNS specificity, acting to promote KCC2 function is a highly promising therapeutic strategy in epilepsy and related disorders. Candidate *KCC2 enhancers* have been proposed based on screening of drug-like compound libraries. However, their mechanisms of action, specificity and therapeutic potential in epilepsy remain unknown. Our project aims to develop a totally novel approach based on molecular modelling and virtual screening to design new molecules with improved specificity and bioavailability. This goal will be achieved by combining advanced medical chemistry approaches (F Acher) together with cellular imaging and electrophysiological techniques (JC Poncer) in order to design and optimize specific KCC2 allosteric modulators, test their potency and specificity as well as evaluate their therapeutic potential in epilepsy.

Context and objective :

Alterations in neuronal chloride regulation is emerging as a hallmark of neurological disorders¹, including epilepsy²⁻⁶, autism spectrum disorders⁷⁻⁹ and neuropathic pain¹⁰⁻¹². Perturbed expression and/or function of the cation-chloride cotransporters (CCCs) KCC2 and NKCC1 are reported in these conditions and expected to impair fast inhibitory signalling mediated by the chloride-permeable GABA_A receptor (GABAAR)¹. Thus, while NKCC1 imports Cl⁻ and K⁺ inside neurons, KCC2 usually contributes to maintain low intraneuronal [Cl⁻], thereby promoting the inhibitory effect of GABAAR activation¹³. Reduced KCC2/NKCC1 functional ratio in the pathology may then depolarize the reversal potential of GABAAR currents and even promote excitatory actions of GABA^{7,10,14-16}. This in turn may contribute to anomalous neuronal activities underlying the pathology. Moreover, several recent studies revealed a variety of effects of KCC2 downregulation, independent of ion transport, including altered glutamatergic synaptic transmission¹⁷ and plasticity¹⁸ as well as enhanced neuronal excitability¹⁹.

Strategies targeting CCC function or expression have therefore been tested in animal models of neurological disorders, including epilepsy. For instance, intracerebral infusion of the NKCC1 antagonist bumetanide was shown to prevent epileptogenesis in an experimental model of temporal lobe epilepsy (TLE)²⁰. However, bumetanide has numerous side-effects - as NKCC1 is also widely expressed in non-neuronal cells - and hardly crosses the blood/brain barrier, making it a poor candidate in CNS disorders²¹. Instead, promoting KCC2 function or expression holds many promises, as KCC2 is almost exclusively expressed in neurons. Thus, viral-based KCC2 overexpression in cortex was recently shown to prevent seizures in mice³. Screening of drug-like compound libraries also identified small molecules suggested to act as *KCC2 enhancers*^{11,22,23}. Despite their well-documented therapeutic benefit in neuropathic pain and other disorders^{11,22-24}, their effect in epilepsy, however, has been overlooked²⁵. In addition, their mechanism(s) of action and specificity remain unknown and even controversial^{26,27}. **An advantageous alternative to the screening of chemical compound libraries consists in**

specifically designing ligands to target effectors by molecular modelling and virtual screening²⁸⁻³⁰. Considerable progress has been made recently in understanding the molecular mechanisms regulating KCC2 function and membrane stability³¹⁻³⁴ as well as in deciphering the 3D structure of CCCs^{6,35,36,37} and some of their key regulators³⁸⁻⁴⁰. This knowledge may then now be used to implement novel approaches of computational chemistry to design specific KCC2 ligands to promote its membrane expression and function.

We propose that **acting to promote KCC2 function represents a highly promising therapeutic strategy in epilepsy and related disorders**. Our preliminary data demonstrate the effectiveness of existing KCC2 enhancers in preventing epileptiform activity *in vitro* and *in vivo*. However, their specificity is limited and other adverse effects are also observed. Therefore, our project aims at i) designing new molecules with improved specificity and bioavailability and ii) evaluating and comparing their therapeutic potential in the context of epilepsy. To this end, an **interdisciplinary approach** will be implemented, combining **advanced computational chemistry and virtual ligand screening** with **biochemical and super-resolution imaging techniques** as well as **electrophysiological recordings** from human brain tissue and animal models.

Specific aims

Aim 1. To model the carboxy-terminal domain of KCC2 interaction with kinases SPAK and OSR1 and identify new candidate KCC2 enhancers by virtual screening

Our modeling approach will aim at designing new molecules to more selectively enhance KCC2 function and stability than existing compounds. KCC2 membrane stability and function are primarily regulated through posttranslational modifications, in particular phosphorylation of residues of its carboxy-terminal domain³¹⁻³⁴. These sites therefore represent targets of choice for designing specific KCC2 enhancers. We will focus on Thr^{906/1007} residues in the carboxy-terminal domain (CTD) that we and others have shown reduce KCC2 membrane stability and function upon phosphorylation by the SPAK and OSR1 kinases³¹⁻³⁴. In order to avoid the lack of selectivity inherent to the highly conserved ATP-binding site of the kinases, we will use molecular modelling and virtual screening to design ligands targeting specific allosteric sites of the kinase or substrate. The recently released cryoEM structure of KCC2 (PDB ID 6m23) reveals residues Thr^{906/1007} to be in a solvent-exposed position. Based on available structures and homology models of SPAK and OSR1^{39,40}, we propose to dock these structures to the KCC2-CTD 3D-structure in order to define selective contact hotspots between them and then prevent these protein-protein interactions. These hotspots will be first validated by mutagenesis and chloride-imaging based functional assays. We will then search for small molecules/peptides with high affinity for these hotspots by virtual high-throughput screening²⁹ using Discovery Studio (Dassault Systemes BIOVIA), routinely used by F. Acher. Curated libraries of commercial small compounds/peptides will be virtually docked at the specific sites of the 3D structures and scored. Top-ranked molecules will then be used for pharmacological evaluation. The validated hits will be then chemically optimized for *in vivo* delivery and stability.

Aim 2. To compare the specificity and modes of action of existing vs. novel KCC2 enhancers in hippocampal neurons *in vitro*

We will test the efficacy and specificity of compounds obtained from Aim 1 and characterize their effects on KCC2 expression/function as well as GABA signaling in cultured rat

hippocampal neurons. Specifically, we will test i) KCC2 membrane expression (surface biotinylation), ii) KCC2 phosphorylation state (phosphosite-specific antibodies⁴¹), iii) KCC2 membrane dynamics (single particle tracking^{31,41}) and iv) KCC2 function (focal GABA uncaging in whole-cell and gramicidin perforated patch recordings^{18,19,41}). Preliminary results from JC. Poncer suggest CLP257 and piperazine derivatives may act similarly to promote KCC2 function through reduced phosphorylation at Thr^{906/1007} and enhanced clustering. We will also explore the specificity of these effects, in particular with respect to NKCC1 and GABAAR function²⁶.

Aim 3. To evaluate and compare the antiepileptic potential of KCC2 enhancers in human epileptic tissue *in vitro* as well as in animal models of temporal lobe epilepsy

Human cerebral tissue resected from patients with pharmaco-resistant epilepsy represent an invaluable model for exploring the effect of candidate antiepileptic drugs *in vitro*. Using multielectrode array recordings⁴², in collaboration with the Neurosurgery department of Sainte-Anne Hospital, we will compare the effect of novel KCC2 enhancers on neuronal activity and spontaneous interictal discharges (IIDs) from slices of human epileptic hippocampus. In parallel, we will test these compounds in the pilocarpine mouse model of TLE. This will let us test the effect of KCC2 enhancers both on epileptogenesis (chronic injection during 8 days post *status epilepticus* (SE)) and during the chronic phase (from day 35 post SE). Using telemetric ECoG and video recordings, we will monitor and quantify seizure frequency and duration as well as IIDs in saline vs. KCC2 enhancer-injected mice. This task based on assays routinely used in the Poncer lab will be performed in collaboration with trained electrophysiologists.

Justification of suitability for *i-Bio*:

This interdisciplinary project gathers two groups with complementary expertise in **medicinal chemistry** and **cellular neurobiology**. It is based on solid preliminary data and expertise from both partners and aims to produce the first proof-of-concept preclinical evidence for using KCC2 enhancers in epilepsy. It is also innovative and ambitious as it proposes to develop a totally novel approach to designing KCC2 enhancers with improved specificity and bioavailability, using state-of-the-art molecular modeling and virtual screening of allosteric antagonists KCC2 phosphorylation. We trust this approach, which has been successfully applied to other receptors, transporters and kinases, should let us identify the most promising molecules for optimal translation to clinical studies, both in epilepsy and other neurological disorders associated with CCC dysfunction. It will also produce invaluable data on KCC2 structure and the understanding of molecular interactions between KCC2 and its regulatory partners, with a predicted impact beyond the field of epilepsy research.

Role of each supervisor / skills provided:

Co-supervisor 1: Jean Christophe Poncer, PhD. Research Director, INSERM. Institut du Fer à Moulin (UMR 1270, INSERM/Sorbonne University, Paris).

His group combines state-of-the-art super-resolution imaging (Lévi/Renner) and electrophysiological approaches, both *in vitro* (patch clamp, multi-electrode arrays) and *in vivo* (telemetric EEG and intracerebral silicon probes). His lab has made important discoveries in the field of cation chloride cotransporters, revealing in particular i) a variety of KCC2 physiological functions independent of ion transport (PNAS 2011, JNeurosci 2015, Cell Rep 2019, JPhysiol 2020) and ii) mechanisms of KCC2 activity-dependent regulation through phosphorylation-induced changes in membrane dynamics (JNeurosci 2013, Nat Comm 2017).

Publications: 42 articles in peer-reviewed journals. H-index 29. 4222 citations.

Co-supervisor 2: Francine Acher, PhD. Research Director, CNRS. Laboratoire de Chimie et Biochimie pharmacologiques et toxicologiques (LCBPT, UMR 8601 CNRS/Univ. de Paris).

Her group has a very solid background in medicinal chemistry. F Acher has been involved in the design and synthesis of non-natural amino acids and the investigation of their roles in various biological systems. Her interdisciplinary research was made possible by close interaction with expert collaborators in pharmacology, molecular biology and behaviour. She discovered potent ligands using virtual HTS of homology models of GPCRs and membrane transporters (Neuron 2008, J Med Chem 2005/2010, JBC 2012). Several of these hits, once chemically optimized, showed beneficial effects in animal models of Parkinson's disease, chronic pain, schizophrenia, addiction and epilepsy (Faseb J 2012, JNeurosci 2013, Psychopharm 2012, Neuropharmacol 2018, Neurobiol Dis 2019). She will be in charge of supervising all the molecular modelling approach and chemical synthesis.

Publications: 130 articles in peer reviewed journals. H-index 34. 11 international patents. 3764 citations.

Profile of the desired student:

The candidate should have a background in synthetic chemistry or pharmaceutical chemistry as well as notions of cellular biology and biochemistry, and be fluent with programming in Matlab or equivalent. Additional training in cellular and molecular biology as well as electrophysiology will be provided through the i-Bio training program. Specific training in molecular modelling and virtual screening tools (Discovery Studio; Dassault Systèmes BIOVIA) will be provided through an active collaboration of F. Acher with Dassault Systèmes.

References

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