UNIVERSITÀ DEGLI STUDI DI PADOVA

PROJECT

Tutor's Name	Girolamo Calo'
Cotutor's Name	Davide Malfacini

1) Project title
Innovative approaches to target the Nociceptin / Orphanin FQ receptor
2)Abstract (max 500 words)
One-third of marketed drugs act at G Protein-Coupled Receptors (GPCRs). The nociceptin/orphanin FQ (N/OFQ) peptide receptor (NOP), is the fourth member of the opioid receptor family and is activated by the endogenous 17-amino acid neuropeptide N/OFQ. Although structurally related to classical opioid receptors, NOP displays distinct pharmacological and functional properties. It is widely expressed in the central nervous system and in peripheral tissues, where it contributes to the regulation of pain transmission, stress responsivity, arousal, sleep, migraine-related pathways, and neuroimmune functions. Evidence also supports a role of the N/OFQ–NOP system in glial and immune modulation, making it an attractive target not only in analgesia, but also in broader pathological settings involving neuroinflammation and altered neuronal excitability. At the signaling level, NOP mainly couples to Gi/Go-family proteins, inhibits cAMP formation, modulates ion channels, and can also engage β-arrestin-dependent pathways. Importantly, different ligands may promote distinct receptor conformations and selectively favor signaling through specific G-protein subtypes or β-arrestin, thus generating biased agonism. This concept is particularly relevant for NOP, because selective engagement of different signaling pathways may translate into differentiated therapeutic effects and improved safety profiles. For this reason, the development of innovative NOP ligands requires not only potency and selectivity, but also a precise understanding of their signaling fingerprints. The Ph.D. student will be involved in a highly collaborative, multidisciplinary, and international project integrating medicinal chemistry, computational modeling, molecular pharmacology, cell biology, and receptor imaging to investigate innovative strategies for targeting the NOP receptor. In particular, we will employ: i) structure-activity relationship (SAR) studies on peptide and non-peptide ligands to identify new NOP ligands with improved pharmacological properties; ii) site-directed mutagenesis of the NOP receptor to validate in silico predictions and define the molecular determinants of ligand binding, efficacy, and signaling bias; iii) a broad range of pharmacological assays to characterize ligand activity at β-arrestin and multiple G-protein-mediated pathways, including bioluminescence-based cAMP GloSensor, TruPath transducer profiling, calcium mobilization approaches with engineered G proteins, and label-free bioimpedance assays; iv) advanced imaging methods to investigate receptor localization, trafficking, and ligand-dependent redistribution using fluorescent probes and live-cell microscopy; and v) the genetically encoded fluorescent biosensor NOPLight to directly monitor receptor activation in cells.

In parallel, the project will address additional aspects of major mechanistic and translational relevance, including vi) the identification of primary cells and native cellular models endogenously expressing NOP, with special attention to neuronal, glial, and immune systems; and vii) the study of receptor homodimerization and heterodimerization, particularly NOP-mu opioid receptor interactions, and their consequences for receptor signaling and pharmacology.

Altogether, this project will provide novel tools and concepts to better understand NOP receptor biology and to support the future development of innovative ligands for disorders such as pain, migraine, insomnia, and neuroimmune dysfunction.