STRUCTURAL AND MECHANISTIC INSIGHTS INTO THE SUBTYPE AND FUNCTIONAL SELECTIVITY OF SEROTONIN RECEPTORS

ABSTRACT

The ongoing revolution in structural biology promoted by methodological enhancements in protein crystallography and, more recently, by technical advances in cryogenic electron microscopy is allowing an unprecedent level of insights into the molecular mechanisms of biological system. G protein-coupled receptors (GPCR) are at the vanguard of these scientific achievements, due to the central roles that they play in human biology and disease. Among them, the serotonin GPCR family has always been particularly appealing, as these receptors regulate innumerous processes throughout the human body, including mood, memory, perception, and cognition – all fundamental aspects of the human psyche that ultimately differentiate humans from the other creatures.

However, just as important as gathering all these structural and biochemical data, is to understand 'what stories is the data telling us', i.e., to combine, compare, and analyze it holistically – literally 'as a whole' – aiming to elucidate the molecular details associated with the functioning of GPCRs. In that sense, this thesis is about four stories that the serotonin receptors structures and related data can tell us about their mechanisms of ligand recognition, binding selectivity, activation, coupling/signaling, and functional selectivity.

In **manuscript 1**, we gather all the available structure information on serotonin receptors and combine it with ligand binding affinity, site-directed mutagenesis, sequence alignment and analysis, plus experimental and predicted binding poses aiming to elucidate the molecular determinants for ligand selectivity within the 12 human serotonin receptor subtypes. All this information is then condensed into a 'roadmap' of proven and predicted selectivity hotspots, which can help the design and synthesis of better drugs, with reduced side effects.

In **manuscript 2**, we board into a *trip* to the chemistry, selectivity, and action of psychedelics, guided by the 5-HT_{2A} receptor, as these drugs resurge as effective therapies for neuropsychiatric disorders. Through computational modelling tools, we show for the first time, how a hydrophobic tunnel explains the binding, selectivity profile and the *in vivo* responses of psychedelic amphetamines and phenethylamines. Apart from shedding light into a half-century standing question, our results also suggest a potential functional selectivity hotspot in the 5-HT_{2A} receptor, which could be exploited to design agonists devoid of hallucinogenic properties.

In **manuscript 3**(*1*), we dig into the mechanisms of receptor regulation by cholesterol and membrane phospholipids guided by the $G_{i/o}$-coupled structures of 5-HT_{1A}, 5-HT_{1D} and 5-HT_{1E}. We also investigate the underlying aspects related to serotonin pan-agonism at its multiple

receptors, despite the modest sequence conservation of orthosteric binding pockets across subtypes, while also examining the molecular basis for the high constitutive activity of 5-HT_{1A} and the selectivity determinants for the triptan class of antimigraine drugs.

In **manuscript 4**, we dive into the G protein-binding interface guided by the G_s-coupled structures of 5-HT_4, 5-HT_6, and 5-HT_7 and a very distinct G_i-coupled structure of 5-HT_4. We find a receptor 'macro-switch' for G_s-over-$G_{i/o}$ protein selectivity related to the differential length between the intracellular portions of TM5 and TM6. This mechanism seems to be universal among class A GPCRs – ultimately encoded in the receptor sequence – and capable of promoting unique sets of receptor-G protein interactions that act as selectivity microswitches.

In summary, this thesis provides new insights into the structural and dynamics mechanisms associated with the functioning and signaling of serotonin GPCRs, from the ligand binding pocket (subtype selectivity) to the G protein binding domain (functional selectivity). These insights can support the design and development of new therapeutical drugs, with improved pharmacological profiles and reduced side effects, while helping advance our molecular understanding of serotonin receptors and, ultimately, our understanding of the human mind.

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1. P. Xu *et al.*, Structural insights into the lipid and ligand regulation of serotonin receptors. *Nature* **592**, 469-473 (2021).