1. State of the art

G protein-coupled receptors (GPCRs) are the largest family of membrane proteins whose role is the transduction of signal from outside to inside the cell. They interact with a variety of bioactive molecules, such as hormones, neurotransmitters, ions, photons, and mediate the signal transduction through G proteins. GPCRs are responsible for the regulation of a great number of physiological processes, such as the mediation of neurotransmission and hormonal action, the transmission of the light and of odorant signal, the cell growth and the immune defense. [1]

All GPCRs share common structural features even if their sequence identity is low. They are all constituted by a central domain of seven transmembrane α-helices (TM1-7), connected by three extracellular (EL1-3) and intracellular (IL1-3) loops, and an extracellular N-terminal and an intracellular C-terminal domain. While the TM helices are well conserved, the intra- and extracellular regions greatly differ in length and function, providing specific properties to each receptor. [2]

GPCRs are divided into five families on the basis of their sequence and structural similarity: rhodopsin (family A), secretin (family B), glutamate (family C), adhesion and Frizzled/Taste2. Family A is the largest and most investigated, it comprises for example adrenergic, serotonergic, dopaminergic, muscarinic, hystaminergic and opioid receptors, which have a notable therapeutic impact. [3]

2. Objectives & expected results

It has been estimated that more than half of all modern drugs are targeted at GPCRs, which appear the most important class of human druggable targets. Indeed, their considerable therapeutic implication inspires great effort in the field of medicinal chemistry. [4]

This PhD project aims to investigate the mechanism of action of known agonists and antagonists of GPCRs using molecular dynamics simulations. The tool “Supervised Molecular Dynamics” (SuMD) will be adopted to rationalize mechanistic information about the interaction between ligand and receptor, which would be useful for lead optimization of binders with better pharmacodynamic properties.

A second objective is the study of the selectivity of a ligand for different receptors exploring its behaviour in presence of more than one GPCR. Afterwards the idea is to test a database of ligands on GPCRs, laying the groundwork for SuMD virtual screening. The idea of performing SuMD high-throughput screening would be of remarkable interest for the pharmaceutical industry, but the exploration of such a big chemical space will require a good parametrization. In fact, a great part of this work will be devoted to the parametrization of the ligands.
In addition, as a preliminary phase it will be necessary to validate the method SuMD by testing GPCRs crystal structures that have been co-crystallized with their ligand as a true positive.

3. Methods

Different techniques in the field of molecular modelling will be exploited in order to achieve the objectives indicated before. Molecular dynamics (MD) simulation techniques will be applied to the GPCRs, that are membrane proteins, as a consequence the biophysics of the lipid membrane has to be taken into account. ACEMD, AMBER and GROMACS will be used. These programs are optimized to utilize the computational power of graphics processing units (GPUs). In particular it will be exploited the software SuMD, which has been implemented by the Molecular Modelling Section. This tool is based on classical MD simulation in which the ligand-receptor approaching distance is supervised by a tabu-like algorithm. This allows the exploration of ligand-receptor recognition pathway in a nanosecond timescale. [5]

The application of molecular dynamics simulation in drug discovery requires the accurate definition of parameters for the description of the molecular system. Proteins have already been parametrized, but the vast diversity of small molecules requires parameter optimization. In this work GAUSSIAN 09 and the force field TOOLKIT will be used for the parametrization of the ligands. [6]

It will be useful to employ the molecular docking methods for the analysis of databases of compounds and as a starting point for MD simulations: the programs PLANTS, GOLD and GLIDE will be used.

Since the crystal structures are available for less than 30 unique GPCRs, it will be necessary to adopt homology modelling techniques for building structure models.

4. Workplan

The work will be organized almost in the following way:
1) METHOD VALIDATION:
the first year will be devoted to the application of the tool SuMD to the GPCRs structures present in the Protein Data Bank that have been co-crystallized with their ligands. In this way it will be possible to evaluate the performance of the tool in the prediction of the binding mode observed in the crystal.

2) AGONISTS AND ANTAGONISTS RECOGNITION MECHANISM:
in the second year SuMD will be exploited to characterize the recognition pathway of agonists and antagonists with the systems previously validated.

3) SELECTIVITY AND DATABASE ANALYSIS:
in the third year simulations of a ligand pathway will be performed in presence of more than one type receptor in the membrane environment, with the purpose to investigate selectivity. In a second phase the screening of a database of ligands will be performed. This work will require a preliminary good parametrization of the ligands.

During the PhD course a period abroad could be useful to improve my knowledge and computational skills.
5. Relevant references


