## Abstract

Computational chemistry represents today a valid and fast tool for the research of new compounds with potential biological activity. The analysis of ligand-macromolecule interactions and the evaluation of possible "binding modes" have a crucial role for the design and the development of new and more powerful drugs. *In silico* Virtual Screening campaigns of large libraries compounds (fragments or drug-like) on a specific target allow the selection of promising compounds, leading the identification of new scaffolds. The accurate analysis and the comparison of different bioactive compounds clarify the molecular basis of their interaction and the construction of pharmacoforic models.

In parallel, another crucial aspect of pharmacological research is the identification of targets of interaction of bioactive molecules, and this is particularly true for compounds from natural sources. In fact, a wide range of drug tests on a large number of biological targets can represent a useful approach for the study of natural products, but often one of the main problems is their limited availability.

Starting from these assumptions, a new computational method named Inverse Virtual Screening is described in details in this thesis. The different works based on this approach were performed considering panels of targets involved in the cancer events, determining the identification of the specific antitumor activity of the natural compounds investigated.

Inverse Virtual Screening studies were performed by means of molecular docking experiments on different natural compounds, organized in small libraries or as single compounds. Firstly, a mathematical method for the exclusion of false positive and false negative results was proposed applying a normalization of the predicted binding energies (expressed in kcal/mol) obtained from the docking calculations. Then this approach was applied on a library of 10 compounds extracted from natural sources, obtaining a good validation through *in vitro* biological tests. Afterwards, another study was performed on the cyclopeptide namalide. Its biological inhibitory activity and selectivity on Carboxipeptidase A target was in accordance with Inverse Virtual Screening results.

Virtual Screening topic was also inspected analyzing the efficacy of Molecular Dynamics-based methods for the accurate calculations of the binding affinities. This work was conducted on a library of 1588 compounds (44 ligands + 1544 decoys) extracted from the DUD database on trypsin target, using the Linear Interaction Energy (LIE) method by means of extensive Molecular Dynamics simulations. Four different LIE results obtained combining different scaling factors were compared with docking results, evaluating and comparing ROC and enrichment curves for each of the considered methods. Poor results were obtained with LIE, and further analysis with MM-GBSA and MM-PBSA approaches are under investigation.

Moreover, *in silico* screenings were performed for the detailed study of natural compounds whose activities are known a priori. With this procedure, several binding modes were reported for a library of compounds on PXR target, whose activity or inactivity were rationalized comparing their binding poses with that of Solomonsterol A, used as a reference compound on this receptor. The presence/absence of biological activity of another library of compounds extracted from the marine sponge *Plakinastrella Mamillaris* on PPAR-γ and for the diterpene oridonin on HSP70 1A are described at a molecular level with molecular docking and Molecular Dynamics simulations.

The putative binding modes for the reported molecules was described offering a complete rationalization of docking results, evaluating how ligand target specific interactions (e.g. hydrophobic, hydrophilic, electrostatic contacts) can influence their biological activity.