Nuclear receptors are among the most important intracellular regulating molecules that convey diverse internal and external signals into regulation of genetic programs. Genetic programming established or modified by these proteins affect virtually all the aspects of life of multicellular organisms. The intensive research on transcriptional regulation and selectivity of nuclear receptors nourished in attempts to decipher the complex network of molecular events involving such molecules. By unraveling the molecular rules, which define their control – both in space and time – over protein-protein and protein-DNA interactions, a myriad of possibilities could be opened up for developing more efficient drugs with superior therapeutical value.

Within the present project, we propose to study nuclear receptors by X-ray crystallography, small angle X-ray scattering (SAXS), biochemical and biophysical methods as well as by biocomputing simulations, in attempt to better understand what conformational changes of the receptor’s 3D structures are induced by its binding to specific ligands (agonists and antagonists) and how this could influence the receptor’s oligomeric state. In addition to those, we are also interested in characterizing its interactions with coregulating proteins (coactivators and corepressors), and also in modifying their stability. We aim at gaining insights into recognition of the nuclear receptors DNA response elements, by a combined approach of protein crystallography, SAXS and fluorescence anisotropy. Finally, we plan to study the role of nuclear receptor dynamics and flexibility in their function, seeking to determine the preferable dissociation pathways of the nuclear receptor ligands from their respective receptors by molecular dynamics simulations and experimental studies of the mobility and solvent accessibility changes by using hydrogen/deuterium exchange combined with mass-spectroscopy. It is important to stress that these aims have an immediate impact on the rational development of hormone agonists and antagonists, which are themselves poised for the scientific and technical development of Brazil in the area of nuclear receptors, and directly related to the interests of molecular biology, medicine and pharmaceutical industry.
The main focus of research consists on structural, biochemical, biophysical and functional studies of nuclear receptors and related proteins. The structures of the ligand binding domain (LBD) of thyroid hormone receptor (TR) were determined and the role of the hinge domain was analyzed based on structural results and functional experiments. In addition, we conducted molecular dynamics computational studies of the escape pathways of ligands from TR LBD. We demonstrated that, contrary to what is being currently accepted in the area, there are at least three dissociation pathways of ligands from TR, and that the “canonic” escape path, via helix 12, is not the preferred one.

Furthermore, by using a combination of biophysical and biochemical techniques we have established for the first time that TR can form tetramers in solution. We reconstructed low-resolution models of TR dimers and tetramers in solution by using small-angle X-ray scattering (SAXS) and then went on to discuss physiological implications of the multimeric forms of thyroid receptor. We determined the X-ray structure of another nuclear receptor, PPARγ LBD complexed with a synthetic ligand derived from cannabinoid acid, which revealed that the ligand binds PPARγ at two binding sites, in the interior and on the surface of LBD. Biophysical studies of NGFI-B by SAXS and hydrogen/deuterium exchange, followed by mass-spectrometry, demonstrated that this orphan nuclear receptor forms dimers through non-canonical dimer interface which is similar, but not identical, to the glucocorticoid receptor (GR) dimer interface. This might explain competition between these two receptors in cells.