Summary

Interactions between small-molecule compounds (ligands) and proteins constitute the foundation of nearly all biological processes and are essential for the proper functioning of living organisms. The ability of a ligand to recognize and bind to a protein depends not only on its physicochemical properties, derived from its specific structure, but also on environmental factors such as pH, temperature, and the presence of competing ligands. Therefore, a thorough understanding of how and why these interactions occur requires a comprehensive analysis of both their thermodynamic properties and their effects on protein structure.

Therefore, elucidating the mechanisms of ligand-protein interactions at the molecular level goes beyond fundamental research. It provides essential insights for the rational design of new compounds with desired properties. This knowledge is particularly valuable in fields such as bioorganic chemistry, pharmacy, and biotechnology. It enables the prediction of molecular behaviour in complex biological systems and supports the design of biologically active compounds.

This PhD thesis aimed to identify the key structural and physicochemical features of ligands that determine the strength of their binding to proteins, as well as the mechanism and location of these interactions. Two complementary yet contrasting model systems were selected: albumins, which play a key role in the transport of endogenous and exogenous compounds, and lysozyme, an enzyme characterized by a different structure and surface charge distribution. Several classes of compounds differing in chemical structure, charge, and physicochemical properties were analyzed, including anionic surfactants (1-alkylsulfates and 1-alkylsulfonates) with varying hydrocarbon chain lengths, tetraphenylborate ions, polyoxovanadates, and hexacyanoferrate(II)/(III) ions. Although the selected ligands are not drugs *per se*, their structures resemble motifs commonly found in biologically relevant compounds, enabling the identification of key features that govern their interactions with proteins.

The study employed isothermal titration calorimetry along with a range of complementary experimental, spectroscopic, and electrochemical techniques. This combination enabled the characterization of ligand-protein interactions at the molecular level and provided insight

into protein structural changes induced by physical and chemical factors. The experimental results were further supported by *in silico* methods, which offered additional understanding of the nature of these interactions and allowed for the identification of potential binding sites on the proteins. This multidisciplinary approach allowed for a systematic analysis of how key structural parameters of ligands influence the mechanism and strength of their interactions with proteins, as well as for the formulation of general principles describing how ligand structure and physicochemical properties govern protein affinity.

The results demonstrate that protein domain organization and structural flexibility play a key role in this process. These features enable the selective binding of ligands with diverse properties, forming the basis of protein biological functions. The results show that it is not a single ligand feature, but rather the synergistic interplay of size, charge, and hydrophilic-hydrophobic character that determines the binding mechanism, ranging from electrostatic interactions, through mixed (electrostatic and hydrophobic) effects, to predominantly hydrophobic interactions.

This comprehensive overview of the interactions between proteins and small-molecule ligands explains the observed phenomena at the molecular level and can serve as a basis for predicting the behavior of such compounds in biological systems, as well as for the rational design of new substances with desired properties.