OVERVIEW

The presented doctoral thesis includes five published scientific articles and one manuscript prepared for submission. All describe studies on three protein models. The four papers numbered (1) – (4) in the list of publications, share a common objective e.i. observing and describing functional protein dynamics and conformation change induced by ligand or substrate binding, and represent the main result of my PhD work.

The papers (1) and (4) offer results of a project from my home laboratory at the Institute of Microbiology AS CR, Laboratory for Biology of Secondary Metabolism, under the supervision of Jiří Janata, PhD. The project is focused on the protein-protein dynamics interaction of mitochondrial processing peptidase (MPP) from *Saccharomyces cerevisiae* with its preproteins substrates.

The papers (2) and (3) describe results of a project, in which I have participated during my Marie Curie fellowship at the Ecole Polytechnique (Palaiseau Cedex, France), Laboratory for Optics and Biosciences, in 2004. The project concerns research on protein structural dynamics of the heme-based oxygen sensor FixL from *Bradyrhizobium japonicum*, in which oxygen binding to the heme sensor domain induces conformation change, which regulates the activity of neighboring kinase domain.

In both projects, analogy in methodical approach, i.e. series of molecular biology and biochemistry techniques, was used; the highly conserved amino acid residue and/or region, which are suggested to play a key role in ligand binding and discrimination, were modified by site directed mutagenesis and the protein mutant forms were purified. The binding dynamics of the mutant forms was examined by various biophysical methods: i) the precursor protein binding to MPP was observed by time-resolved and steady-state fluorescence spectroscopy ii) the dynamics of the oxygen binding to the FixL heme domain was demonstrated by femtosecond transient absorption spectroscopy and time-resolved resonance Raman spectroscopy. Finally, the molecular dynamics simulations were performed in both projects to gain insight into the mechanism of the ligand binding, recognition and ligand specificity at a molecular level.

The publications (5) and (6) give the results of another project from the Laboratory for Biology of Secondary Metabolism – a study on proteins involved in the biosynthesis of secondary metabolites. Although the used methodical approach was similar to those of the above mentioned projects, the aims of the experiments were different from the observing the protein dynamics. Thus, these papers are summarized very briefly.

The present thesis reports two examples of a protein dynamics of different extents and time scales:

- In the case of **mitochondrial processing peptidase** we studied the domain motion as well as local loop motion on the nanosecond time scale.
- On the other hand, the change of the conformation of the heme as the prosthetic group of **FixL protein** was observed on the picosecond and/or subpicosecond time scale.