Cytochromes P-450 (P450s) belong to haemoprotein superfamily and they are responsible for metabolism of a wide variety of compounds, among others many drugs and carcinogens. P450s serve as the terminal oxidases in the mixed function oxidase system in cooperation with a redox partner NADPH: cytochrome P450 reductase (CPR) providing input of two electrons to the reaction cycle of P450. The CPR can be substituted by other redox partner of P450, cytochrome b5 (cyt b5), to deliver the second electron. Three dimensional structure of P450 is required in order to fully understand its reaction mechanism. At the present time, a homology model of cytochrome P-450 2B4 (CYP 2B4) is available in our laboratory.

In this study, the mapping of interaction domain between CYP 2B4 and cyt b5 employing a crosslinking agent EDC to form amide bonds between close complementary charged amino acid side chains was the first goal. We have identified five interacting amino acid pairs in total using mass spectrometry (MS).

The second research interest was to verify and refine the CYP 2B4 model using a photoaffinity labelling with N-(p-azidobenzyl)-N-methyl-p-aminophenylamine probe. This photoreactive probe is known as CYP 2B4 ligand binding to the central iron atom of haem. After photoactivation the arginine 197 was found by MS technique as a target aminoacid residue for reactive intermediate of this probe.

Both presented results validate our methodology approaches and elucidate the molecular mechanism of CYP 2B4-ligand or CYP 2B4-cyt b5 interaction. (In Czech)