Abstract

A comprehensive understanding of physiological role of proteins requires knowledge of their three-dimensional structure, dynamics and protein-protein interactions. Chemical cross-linking in combination with mass spectrometry represents an alternative approach to standard methods for protein structure elucidation (X-ray crystalography, NMR spectroscopy) and enables characterization of interaction interface within protein complexes in their native states. The presented thesis is mainly focused on novel cross-linking methodology based on the *in vivo* incorporation of methionine analog with photo-reactive functional group (photo-Met) into the sequence of studied protein (so called protein photo-nanoprobe).

Interaction between two molecules of 14-3-3zeta protein was used as a model to test and optimize the protein photo-nanoprobe production. The findings confirmed usefulness of this approach for mapping the protein-protein interactions. The photo-initiated cross-linking was used to detect the heterooligomeric membrane structures of cytochromes P450 2B4 and b5 and the molar ratio of cytochromes within individual complexes was assessed. The chemical cross-linking in combination with mass spectrometry was employed to characterize the interaction of their catalytic domains and two mutual orientations of cytochromes were determined. Thus,the contact surface regions between membrane hemoproteins were studied.