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Microcrystallization and structural studies of proteins by serial crystallography and x-ray scattering

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Abstract

Proteins are the most complex and versatile biomolecules known, with essential roles in all cellular and physiological processes. They constitute the main structural components of cells, catalyze most biochemical reactions, regulate gene expression, modulate immune responses, and form tissues. Proteins are composed of amino acids, which also have diverse functions as neurotransmitters, hormones, and regulators of various physiological pathways. Proteins have evolved and adapted their structures and functions over billions of years of evolutionary history. This thesis describes methods for expression, purification, and crystallization of proteins, as well as the techniques for analysis and characterization of protein structures at room temperature. This thesis also reports the results obtained from these methods and techniques. We applied various methods to obtain high-quality microcrystals for serial crystallography. We used serial crystallography to determine the structures of human Cytochrome P450 3A4 (CYP3A4) and soluble Epoxide Hydrolase bound to seven ligands. We detected a temperature-dependent difference in the binding mode of one ligand and improved resolution of some flexible loops at ambient temperature compared to cryo-temperature structures. (Paper I & II) We used time-resolved serial femtosecond crystallography to study the structural dynamics of ba_3-type Cytochrome *c* oxidase (C*c*O) after CO photodissociation from reduced heme a_3. We found that CO is stabilized at Cu_B by a transient water molecule, unlike in aa_3-type CcO. This explained the long-lived Cu_B – CO complex and the high oxygen affinity of ba_3-type CcO (Paper III). We confirmed CYP3A4 ligand binding by UV-vis absorbance spectroscopy, showed that CYP3A4 forms tetramers in solution and undergoes a distinct conformational change upon ketoconazole binding by X-ray scattering (Paper IV).