Fluorescence correlation spectroscopy on electron transfer reactions : probing inter- and intramolecular redox processes

Sen, S.

Fluorescence Correlation Spectroscopy on Electron Transfer Reactions

Probing Inter-and Intramolecular Redox Processes

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 30 juni 2016
klokke 13:45 uur

door

Saptaswa Sen

geboren te Kolkata, India
in 1984

Promotoren:	Prof. dr. G. W. Canters (Universiteit Leiden)
	Prof. dr. T. J. Aartsma (Universiteit Leiden)
Promotiecommisie:	Prof. dr. E. R. Eliel (Universiteit Leiden)
	Prof. dr. E. J. J. Groenen (Universiteit Leiden)
	Prof. dr. A. Rowan (Universiteit Nijmegen)
	Dr. M. Huber (Universiteit Leiden)
	Dr. L. Jeuken (Leeds University)

Casimir PhD Series, Delft-Leiden 2016-17

ISBN: 978-90-8593-258-1

The research described in this thesis was financially supported by NWO (Netherlands Organization for Scientific Research).

Contents

Prologue

This is where it all started. I was a research-fellow in Prof. Dr. Shyamalava Mazumdar's group at the department of chemistry, Tata Institute of Fundamental Research (TIFR), Mumbai, India. This group had a very long history of studying metal-containing proteins e.g. cytochrome c_{552}, thermostable cytochrome P450, Cu_A from cytochrome c oxidase and so on. A special focus was on cytochrome $P450_{cam}$, a bacterial mono-oxygenase containing iron in the active site of the protein, the crucial model of my project. The main task was to engineer mutations in the active site of the cytochrome P450 protein, which in turn, could help in the catalytic degradation of pesticides and also of flavonoid compounds. My approach was first to scan available literature to create a full list of plausible mutants. Some of the candidates were removed from the list as they were judged to affect the active site structure of the protein too severely. Then, the rest were evaluated by energy minimization or docking procedures. In the end, I identified a few single and double mutants, which were considered capable of performing catalyzed degradation. Working with something as fragile and complex as a protein really kindled my interest in protein structure-function relationships, enzyme mechanisms and redox chemistry of proteins. I wanted to follow this interest to understand, think and research better. After the completion of the research project on cytochrome P450 enzyme in TIFR, I was lucky to be able to pursue my interests further by becoming a graduate student in Prof. Dr. Gerard W. Canters' group in the department of molecular biophysics at Leiden University, The Netherlands.