



Universiteit
Leiden
The Netherlands

Transient complexes of haem proteins

Volkov, O.M.

Citation

Volkov, O. M. (2007, February 28). *Transient complexes of haem proteins*. Leiden Institute of Chemistry/MetProt Group, Faculty of Mathematics and Natural Sciences, Leiden University. Retrieved from <https://hdl.handle.net/1887/11002>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/11002>

Note: To cite this publication please use the final published version (if applicable).

Transient Complexes of Haem Proteins

PROEFSCHRIFT

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van de Rector Magnificus Prof. Mr. Dr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op woensdag 28 februari 2007
klokke 13.45 uur

door

Oleksandr Mykolayovych Volkov

Geboren te Kiev in 1978

Promotiecommissie

Promotor:	Prof. Dr. G. W. Canters
Co-promotor:	Dr. M. Ubbink
Referent:	Prof. Dr. J. J. Led (Københavns Universitet)
Overigen leden:	Prof. Dr. J. Brouwer Prof. Dr. H. J. M. de Groot Dr. A. M. J. J. Bonvin (Universiteit Utrecht)

Моей маме

Cover image: Structural representation of the protein complex between cytochrome *c* peroxidase (green ribbon) and cytochrome *c* (red ribbon). The blue and gold "clouds" indicate the areas avoided and visited, respectively, by cytochrome *c* in the dynamic state of the complex. Image courtesy of Dr. A. W. J. W. Tepper (Phantatomix Molecular Graphics, Leiden University, Leiden, The Netherlands).

Printed by PrintPartners Ipskamp, Enschede.

ISBN 978-90-9021558-7

2007, Alexander Volkov

Table of Contents

Abbreviations	6
Chapter I	7
Introduction	
Chapter II	23
The highly dynamic complex of cytochrome <i>c</i> and cytochrome <i>b₅</i>	
Chapter III	47
Solution structure of the complex between yeast cytochrome <i>c</i> and cytochrome <i>c</i> peroxidase	
Chapter IV	69
Dynamics in the complex of yeast cytochrome <i>c</i> and cytochrome <i>c</i> peroxidase	
Chapter V	81
Control of the specificity in the transient complex of yeast cytochrome <i>c</i> and cytochrome <i>c</i> peroxidase	
Chapter VI	103
Increased affinity in the complex of yeast cytochrome <i>c</i> and cytochrome <i>c</i> peroxidase	
Concluding Remarks	121
Appendices	129
References	153
Summary	169
List of Publications	180
Biography	181
Acknowledgements	182

Abbreviations

AIR	ambiguity-driven intermolecular restraints
Amp	ampicillin
Cyt <i>b</i> ₅	soluble part of the bovine liver microsomal cytochrome <i>b</i> ₅
Cyt <i>c</i>	<i>iso</i> -1-cytochrome <i>c</i> from yeast, unless mentioned otherwise
CcP	yeast cytochrome <i>c</i> peroxidase
EDTA	N,N,N',N'-ethylenediaminetetraacetic acid disodium salt
EPR	electron paramagnetic resonance
ET	electron transfer
FPLC	fast protein liquid chromatography
HSQC	heteronuclear single-quantum coherence
IPTG	isopropyl- β -D-thiogalactopyranoside
ITC	isothermal titration calorimetry
LB	Luria – Bertani medium
MTS	(1-acetyl-2,2,5,5-tetramethyl-3-pyrroline-3-methyl)-methanethiosulfonate
MTSL	(1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-methyl)-methanethiosulfonate
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser enhancement spectroscopy
P _i	inorganic phosphate
Pr	haem propionate
PRE	paramagnetic relaxation enhancement
rmsd	root-mean-square deviation
RT	room temperature
SB	Super Broth
TB	Terrific Broth
TSP	3-(trimethylsilyl)-propionic acid-D ₄ , sodium salt
TOCSY	total correlation spectroscopy
Tris-HCl	tris(hydroxymethyl)aminomethane hydrochloride
UV-vis	ultra-violet – visible spectroscopy
wt	wild type