

University of Groningen

Robust monooxygenase biocatalysts

Fürst, Maximilian

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2019

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Fürst, M. (2019). *Robust monooxygenase biocatalysts: discovery and engineering by computational design*. University of Groningen.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.



university of
 groningen

Robust Monooxygenase Biocatalysts

Discovery and Engineering by Computational Design

Maximilian J.L.J. Fürst

Cover design and layout: Maximilian Fürst
Printed by Ipskamp Drukkers B.V.

The research described in this thesis was carried out at the “Groningen Biomolecular Sciences and Biotechnology Institute” of the University of Groningen.

The research for this work has received funding from the European Union (EU) project ROBOX (grant agreement n° 635734) under EU’s Horizon 2020 Programme Research and Innovation actions H2020-LEIT BIO-2014-1.

ISBN (printed version): 978-94-028-1563-4

ISBN (electronic version): 978-94-034-1795-0



university of
 groningen

Robust Monooxygenase Biocatalysts

Discovery and Engineering by Computational Design

PhD Thesis

to obtain the degree of PhD at the
 University of Groningen
 on the authority of the
 Rector Magnificus Prof. E. Sterken
 and in accordance with
 the decision by the College of Deans.

This thesis will be defended in public on

Friday 21 June 2019 at 11.00am

by

Maximilian Josef Ludwig Johannes Fürst

born on 10 August 1988
 in Neumarkt i.d.OPf., Germany

Supervisor

Prof. M.W. Fraaije

Co-supervisor

Prof. D.B. Janssen

Assessment committee

Prof. Dr. N.S. Scrutton

Prof. Dr. W.J.H. van Berkel

Prof. Dr. G.J. Poelarends

TABLE OF CONTENTS

Section 1 Introduction	1
Chapter 1: Beyond Active Site Residues: Overall Structural Dynamics Control Catalysis in Flavin- and Heme-Containing Monooxygenases.....	2
Abstract.....	3
Introduction.....	4
Flavoprotein monooxygenases.....	4
Cytochrome P450s.....	10
Conclusions.....	14
Acknowledgements.....	14
References.....	15
Chapter 2: Baeyer-Villiger Monooxygenases: Tunable Biocatalysts for Oxidative Chemistry.....	18
Abstract.....	19
Introduction.....	20
The Baeyer-Villiger reaction of peroxides and monooxygenases.....	21
Sequences and structures.....	23
Mechanism of the Baeyer-Villiger reaction.....	25
Promiscuous catalytic activities.....	28
Enzyme engineering.....	30
Concluding remarks.....	38
References.....	38
Section 2 Robust and Self-Sufficient P450 Monooxygenases	51
Chapter 3: Exploring the Biocatalytic Potential of a Self-Sufficient Cytochrome P450 from <i>Thermothelomyces thermophila</i>	52
Abstract.....	53
Introduction.....	54
Results and discussion.....	56
Conclusion.....	63
Materials and methods.....	63
Acknowledgements.....	66
References.....	66
Section 3 Towards Robust BVMOs	69

Chapter 4: Polycyclic Ketone Monooxygenase from the Thermophilic Fungus <i>Thermothelomyces thermophila</i> : A Structurally Distinct Biocatalyst for Bulky Substrates	70
Abstract	71
Introduction.....	72
Results and discussion.....	72
Conclusions.....	79
Materials and methods	79
Acknowledgements	84
References.....	84
Chapter 5: A Computational Library Design Protocol for Rapid Improvement of Protein Stability - FRESCO.....	88
Abstract	89
Introduction.....	90
Materials.....	92
Methods.....	93
Acknowledgements	104
References.....	105
Chapter 6: Experimental Protocols for Generating Focused Mutant Libraries and Screening for Thermostable Proteins.....	106
Abstract	107
Introduction.....	108
Single mutants generation.....	109
Combining mutations	125
Summary and conclusions.....	137
Acknowledgements	138
References.....	139
Chapter 7: Stabilization of Cyclohexanone Monooxygenase by Computational and Experimental Library Design.....	142
Abstract	143
Introduction.....	144
Results.....	146
Conclusions.....	154
Materials and methods	154
Acknowledgements	157

References	158
Section 4 Understanding Substrate Selectivity and Product Specificity of BVMOs	161
Chapter 8: Side-Chain Pruning Has Limited Impact on Substrate Preference in a Promiscuous Enzyme	162
Abstract.....	163
Introduction	164
Results	166
Discussion.....	176
Materials and methods.....	178
Acknowledgements.....	182
References	183
Chapter 9: Stipulating the Enantio- and Regioselectivity of Enzymatic Baeyer-Villiger Oxidations by Directed Evolution.....	188
Abstract.....	189
Introduction	190
Enantioselectivity.....	192
Regioselectivity.....	203
Conclusions	209
Materials and methods.....	210
References	217
Conclusions and Future Outlook	222
Conclusions?.....	223
Biocatalysis to the rescue?.....	223
Cytochrome P450s—what lurks in the shadow of the king of catalysis?.....	224
Baeyer-Villiger monooxygenases—is the field exhausted?.....	224
References	225
Supporting Information	226
Supporting Figures	227
Chapter 3.....	227
Chapter 4.....	232
Chapter 7.....	251
Chapter 8.....	256
Chapter 9.....	259
Supporting Schemes.....	264

Chapter 9.....	264
Supporting Tables.....	265
Chapter 4.....	265
Chapter 7.....	268
Chapter 8.....	269
Chapter 9.....	272
Supporting References.....	277
Nederlandse Samenvatting	278
Deutsche Zusammenfassung	286
Curriculum Vitae	294
List of Publications	295
Acknowledgments/Danksagung	297



SECTION 1

INTRODUCTION