

Contents

List of Contributors XIII

Preface XXI

- 1 **Directed Evolution of Ligninolytic Oxidoreductases: from Functional Expression to Stabilization and Beyond** 1
Eva Garcia-Ruiz, Diana M. Mate, David Gonzalez-Perez, Patricia Molina-Espeja, Susana Camarero, Angel T. Martínez, Antonio O. Ballesteros, and Miguel Alcalde
 - 1.1 Introduction 1
 - 1.2 Directed Molecular Evolution 1
 - 1.3 The Ligninolytic Enzymatic Consortium 3
 - 1.4 Directed Evolution of Laccases 6
 - 1.4.1 Directed Evolution of Low-Redox Potential Laccases 7
 - 1.4.2 Directed Evolution of Medium-Redox Potential Laccases 7
 - 1.4.3 Directed Evolution of Ligninolytic High-Redox Potential Laccases (HRPLs) 8
 - 1.5 Directed Evolution of Peroxidases and Peroxygenases 11
 - 1.6 *Saccharomyces cerevisiae* Biomolecular Tool Box 15
 - 1.7 Conclusions and Outlook 16
 - Acknowledgments 17
 - Abbreviations 17
 - References 18
- 2 **New Trends in the *In Situ* Enzymatic Recycling of NAD(P)(H) Cofactors** 23
Erica Elisa Ferrandi, Daniela Monti, and Sergio Riva
 - 2.1 Introduction 23
 - 2.2 Recent Advancements in the Enzymatic Methods for the Recycling of NAD(P)(H) Coenzymes and Novel Regeneration Systems 24
 - 2.2.1 *In Situ* Regeneration of Reduced NAD(P)H Cofactors 24
 - 2.2.1.1 Formate Dehydrogenase and Glucose Dehydrogenase 24
 - 2.2.1.2 Phosphite Dehydrogenase 26
 - 2.2.1.3 Hydrogenase 27

2.2.1.4	Glucose 6-Phosphate Dehydrogenase	29
2.2.1.5	Alcohol Dehydrogenase	29
2.2.2	<i>In Situ</i> Regeneration of Oxidized NAD(P) ⁺ Cofactors	31
2.2.2.1	Lactate Dehydrogenase	31
2.2.2.2	NAD(P)H Oxidase	32
2.2.2.3	Alcohol Dehydrogenase	34
2.2.2.4	Mediator-Coupled Enzyme Systems	35
2.3	Conclusions	37
	Acknowledgments	38
	References	38
3	Monooxygenase-Catalyzed Redox Cascade Biotransformations	43
	<i>Florian Rudroff and Marko D. Mihovilovic</i>	
3.1	Introduction	43
3.1.1	Scope of this Chapter	43
3.1.2	Enzymatic Oxygenation	43
3.1.3	Effective Cofactor Recycling	44
3.1.4	<i>In Vitro</i> Multistep Biocatalysis	46
3.1.5	Combined <i>In Vitro</i> and <i>In Vivo</i> Multistep Biocatalysis	48
3.1.6	<i>In Vivo</i> Multistep Biocatalysis	51
3.1.7	Chemo-Enzymatic Cascade Reactions	56
3.1.8	Conclusion and Outlook	60
	References	61
4	Biocatalytic Redox Cascades Involving ω-Transaminases	65
	<i>Robert C. Simon, Nina Richter, and Wolfgang Kroutil</i>	
4.1	Introduction	65
4.2	General Features of ω -Transaminases	66
4.2.1	Cascades to Shift the Equilibrium for Amination	67
4.3	Linear Cascade Reactions Involving ω -Transaminases	69
4.3.1	Redox and Redox-Neutral Cascade Reactions	70
4.3.2	Carbonyl Amination Followed by Spontaneous Ring Closure	75
4.3.3	Deracemization of Racemic Amines Employing Two ω -Transaminases	78
4.3.4	Cascade Reactions of ω -TAs with Lyases and C–C Hydrolases/Lipases	80
4.4	Concluding Remarks	82
	References	83
5	Multi-Enzyme Systems and Cascade Reactions Involving Cytochrome P450 Monooxygenases	87
	<i>Vlada B. Urlacher and Sebastian Schulz</i>	
5.1	Introduction	87
5.1.1	Multistep Cascade Reactions	87
5.1.2	Cytochrome P450 Monooxygenases	88
5.1.3	General Overview of presented cascade types	91

5.2	Physiological Cascade Reactions Involving P450s	92
5.2.1	Multistep Oxidations Catalyzed by a Single P450	92
5.2.2	Multistep Oxidations Catalyzed by Multiple P450s	102
5.3	Artificial Cascade Reactions Involving P450s	108
5.3.1	Cascade Reactions Involving P450s and Cofactor Regenerating Enzymes	108
5.3.1.1	Cofactor Regeneration in Cell-Free Systems (<i>In Vitro</i>)	108
5.3.2	Cofactor Regeneration in Whole-Cell Biocatalysts	114
5.3.3	Artificial Enzyme Cascades Involving P450s and Other Enzymes	115
5.3.3.1	Artificial Multi-Enzyme Cascades with Isolated Enzymes	116
5.3.3.2	Artificial Multi-Enzyme Cascades <i>In Vivo</i>	120
5.4	Conclusions and Outlook	124
	References	125
6	Chemo-Enzymatic Cascade Reactions for the Synthesis of Glycoconjugates	133
	<i>Ruben R. Rosencrantz, Bastian Lange, and Lothar Elling</i>	
6.1	Introduction	133
6.1.1	Impact of Glycoconjugates and Their Synthesis	133
6.1.2	Biocatalysts for the Synthesis of Glycoconjugates	134
6.1.2.1	Glycosyltransferases	134
6.1.2.2	Glycosidases and Glycosynthases	136
6.1.3	Definition of Cascade Reactions	137
6.2	Sequential Syntheses	139
6.2.1	Nucleotide Sugars	139
6.2.2	Glycoconjugates	141
6.3	One-Pot Syntheses	146
6.3.1	Nucleotide Sugars	146
6.3.2	Glycan Structures	148
6.4	Convergent Syntheses	151
6.5	Conclusion	153
	Acknowledgment	153
	References	153
7	Synergies of Chemistry and Biochemistry for the Production of β-Amino Acids	161
	<i>Josefa María Clemente-Jiménez, Sergio Martínez-Rodríguez, Felipe Rodríguez-Vico, and Francisco Javier Las Heras-Vázquez</i>	
7.1	Introduction	161
7.2	Dihydropyrimidinase	163
7.3	N-Carbamoyl- β -Alanine Amidohydrolase	166
7.4	Bienzymatic System for β -Amino Acid Production	173
7.5	Conclusions and Outlook	174
	Acknowledgments	174
	References	174

8	Racemizable Acyl Donors for Enzymatic Dynamic Kinetic Resolution 179
	<i>Davide Tessaro</i>
8.1	Introduction 179
8.2	The Tools 180
8.2.1	The Enzymes 180
8.2.2	The Racemization of Acyl Compounds 182
8.3	Applications of DKR to Acyl Compounds 183
8.3.1	Base-Catalyzed Racemization 183
8.3.2	DKR of Oxoesters 185
8.3.3	DKR of Thioesters 188
8.4	Conclusions 193
	Acknowledgments 194
	References 194
9	Stereoselective Hydrolase-Catalyzed Processes in Continuous-Flow Mode 199
	<i>Zoltán Boros, Gábor Hornyánszky, József Nagy, and László Poppe</i>
9.1	Introduction 199
9.1.1	General Remarks on Reactions in Continuous-Flow Systems 199
9.1.1.1	Stereoselective Reactions in Continuous Flow Systems 202
9.1.1.2	Analytical Applications 203
9.1.2	Nonstereoselective Enzymatic Processes 204
9.2	Enzyme-Catalyzed Stereoselective Reactions in Continuous-Flow Systems 204
9.2.1	Stereoselective Processes Catalyzed by Nonhydrolytic Enzymes 204
9.2.2	Stereoselective Processes Catalyzed by Hydrolases 207
9.2.2.1	Applicable Types of Selectivities 207
9.2.2.2	Stereoselective Hydrolytic Reactions 207
9.2.2.3	Stereoselective Acylations 211
9.2.2.4	Effects of the Operation Conditions and the Mode of Enzyme Immobilization 220
9.3	Outlook and Perspectives 222
	References 222
10	Perspectives on Multienzyme Process Technology 231
	<i>Paloma A. Santacoloma and John M. Woodley</i>
10.1	Introduction 231
10.2	Multienzyme System Classification 233
10.3	Biocatalyst Options 233
10.3.1	Transport Limitations 235
10.3.2	Compartmentalization 237
10.4	Reactor Options 237

10.5	Process Development	239
10.5.1	Recombinant DNA Technology	240
10.5.2	Process Engineering	241
10.6	Process Modeling	241
10.7	Future	244
10.8	Concluding Remarks	245
	References	245
11	Nitrile Converting Enzymes Involved in Natural and Synthetic Cascade Reactions	249
	<i>Ludmila Martíňková, Andreas Stolz, Fred van Rantwijk, Nicola D'Antona, Dean Brady, and Linda G. Otten</i>	
11.1	Introduction	249
11.2	Natural Cascades	250
11.2.1	Nitrile Hydratase – Amidase	250
11.2.2	Aldoxime Dehydratase–Nitrile Hydratase–Amidase	255
11.2.3	Other Natural Cascades	256
11.3	Artificial Cascades	257
11.3.1	Nitrile Hydratase–Amidase	257
11.3.2	Nitrilase–Amidase	258
11.3.3	Hydroxynitrile Lyase–Nitrilase	259
11.3.4	Hydroxynitrile Lyase–Nitrilase–Amidase	261
11.3.5	Hydroxynitrile Lyase–Nitrile Hydratase	261
11.3.6	Oxygenase–Nitrilase	262
11.3.7	Lipase–Nitrile Hydratase–Amidase	263
11.4	Conclusions and Future Use of These Enzymes	264
	Acknowledgments	265
	References	265
12	Mining Genomes for Nitrilases	271
	<i>Ludmila Martíňková</i>	
12.1	Strategies in Nitrilase Search	271
12.2	Diversity of Nitrilase Sequences	272
12.2.1	Nitrilases in Bacteria	274
12.2.2	Nitrilases in Fungi	274
12.2.3	Nitrilases in Plants	275
12.3	Structure–Function Relationships	275
12.3.1	Sequence Clustering	275
12.3.2	Analysis of Specific Regions	276
12.3.3	Analysis of Enzyme Mutants	276
12.4	Enzyme Properties and Applications	277
12.4.1	Arylacetonitrilases	277
12.4.2	Aromatic Nitrilases	278
12.4.3	Aliphatic Nitrilases	278
12.4.4	Cyanide-Transforming Enzymes	279

12.5	Conclusions	279
	Acknowledgment	279
	References	280
13	Key-Study on the Kinetic Aspects of the <i>In Situ</i> NHase/AMase Cascade System of <i>M. imperiale</i> Resting Cells for Nitrile Bioconversion	283
	<i>Laura Cantarella, Fabrizia Pasquarelli, Agata Spera, Ludmila Martínková, and Maria Cantarella</i>	
13.1	Introduction	283
13.2	The Temperature Effect on the NHase–Amidase Bi-Enzymatic Cascade System	284
13.3	Effect of Nitrile Concentration on NHase Activity and Stability	287
13.4	Effect of Nitrile on the AMase Activity and Stability	289
13.5	Concluding Remarks	293
	Acknowledgments	293
	References	293
14	Enzymatic Stereoselective Synthesis of β-Amino Acids	297
	<i>Varsha Chhibra, Moira Bode, Kgama Mathiba, and Dean Brady</i>	
14.1	Introduction	297
14.2	Preparation of β -Amino Acids	298
14.2.1	Chemical Methods for Generating β -Amino Acids	298
14.2.2	Biocatalytic Preparation of Enantiopure β -Amino Acids	299
14.2.2.1	Lipases and Aminoacylases	299
14.2.2.2	Transaminases	300
14.2.2.3	Nitrile Converting Biocatalysts	300
14.3	Nitrile Hydrolysis Enzymes	301
14.3.1	Nitrilase	301
14.3.1.1	Nitrilase Structure and Mechanism	301
14.3.1.2	Nitrilase Substrate Selectivity	302
14.3.2	Nitrile Hydratase	302
14.3.2.1	Nitrile Hydratase Structure and Mechanism	303
14.3.3	Amidases	304
14.3.3.1	Amidase Structure and Mechanism	304
14.3.4	Nitrile Hydratase and Amidase Cascade Substrate Selectivity	304
14.4	Conclusion	308
	Acknowledgments	309
	References	309
15	New Applications of Transketolase: Cascade Reactions for Assay Development	315
	<i>Laurence Hecquet, Wolf-Dieter Fessner, Virgil Hélaine, and Franck Charmantray</i>	
15.1	Introduction	315
15.2	Cascade Reactions for Assaying Transketolase Activity <i>In Vitro</i>	317

15.2.1	Coupling with Other Enzymes as Auxiliary Agents	317
15.2.1.1	Coupling with NAD(H)-Dependent Dehydrogenases	317
15.2.1.2	Coupling with Bovine Serum Albumin	319
15.2.1.3	Coupling with BSA and Polyphenol Oxidase	321
15.2.2	Coupling with a Nonprotein Auxiliary Agent	325
15.2.2.1	Chemoenzymatic Cascade Reaction Based on Redox Chromophore	325
15.2.2.2	Phenol Red as pH Indicator	326
15.3	Cascade Reactions for Assaying Transketolase Activity by <i>In Vivo</i> Selection	329
15.3.1	Biocatalyzed Synthesis of Probes 16a,b	330
15.3.2	<i>In Vitro</i> Studies with Wild-Type TK and Probes 16a,b by LC/MS	330
15.3.3	Detection of TK Activity in <i>E. coli</i> Auxotrophs from Amino Acid Precursors	331
15.4	Conclusion	334
	References	335
16	Aldolases as Catalyst for the Synthesis of Carbohydrates and Analogs	339
	<i>Pere Clapés, Jesús Joglar, and Jordi Bujons</i>	
16.1	Introduction	339
16.2	Iminocyclitol and Aminocyclitol Synthesis	340
16.3	Carbohydrates and Other Polyhydroxylated Compounds	351
16.4	Conclusions	355
	Acknowledgments	356
	References	356
17	Enzymatic Generation of Sialoconjugate Diversity	361
	<i>Wolf-Dieter Fessner, Ning He, Dong Yi, Peter Unruh, and Marion Knorst</i>	
17.1	Introduction	361
17.2	A Generic Strategy for the Synthesis of Sialoconjugate Libraries	363
17.2.1	Synthesis of Sialic Acid Diversity	368
17.2.1.1	Neuraminic Acid Aldolase	368
17.2.1.2	Neuraminic Acid Synthase	371
17.2.2	Nucleotide Activation of Sialic Acids	372
17.2.2.1	Kinetics of Sialic Acid Activation	373
17.2.2.2	Substrate Binding Model	373
17.2.2.3	Engineering of Promiscuous CSS Variants	376
17.2.3	Sialic Acid Transfer	377
17.3	Cascade Synthesis of neo-Sialoconjugates	378
17.3.1	Choice of Sialyl Acceptor	378
17.3.2	One-Pot Two-Step Cascade Reactions	379
17.3.3	One-Pot Three-Step Cascade Reactions	383
17.3.4	Metabolic Diversification	385
17.3.5	Post-Synthetic Diversification	386

17.3.6	Biomedical Applications of Sialoconjugate Arrays	388
17.4	Conclusions	388
	Acknowledgments	389
	References	389
18	Methyltransferases in Biocatalysis	393
	<i>Ludger Wessjohann, Martin Dippe, Martin Tengg, and Mandana Gruber-Khadjawi</i>	
18.1	Introduction	393
18.2	SAM-Dependent Methyltransferases	395
18.2.1	Substrates	396
18.2.2	Cofactors	400
18.2.3	Higher Homologs and Derivatives of SAM	403
18.2.4	Cofactor (Re)Generation	406
18.2.5	Cascade Applications	410
18.3	Conclusion and Outlook	415
	Abbreviations	417
	Acknowledgement	417
	References	418
19	Chemoenzymatic Multistep One-Pot Processes	427
	<i>Harald Gröger and Werner Hummel</i>	
19.1	Introduction: Why Chemoenzymatic Cascades and Why One-Pot Processes?	427
19.2	Concepts of Chemoenzymatic Processes	427
19.3	Combination of Substrate Isomerization and their Derivatization with Chemo- and Biocatalysts Resulting in Dynamic Kinetic Resolutions and Related Processes	429
19.4	Combination of Substrate Synthesis (Without Isomerization) and Derivatization Step(s)	438
19.4.1	One-Pot Processes with an Initial Biocatalytic Step, Followed by Chemocatalysis or a Noncatalyzed Chemical Process	439
19.4.2	One-Pot Process with an Initial Chemo Process, Followed by Biocatalysis	443
19.4.2.1	Combination of Noncatalyzed Organic Reactions and Biocatalysis	443
19.4.2.2	Combination of Metal Catalysis and Biocatalysis	445
19.4.2.3	Combination of Organocatalysis and Biocatalysis	449
19.5	Conclusion and Outlook	453
	References	453
	Index	457