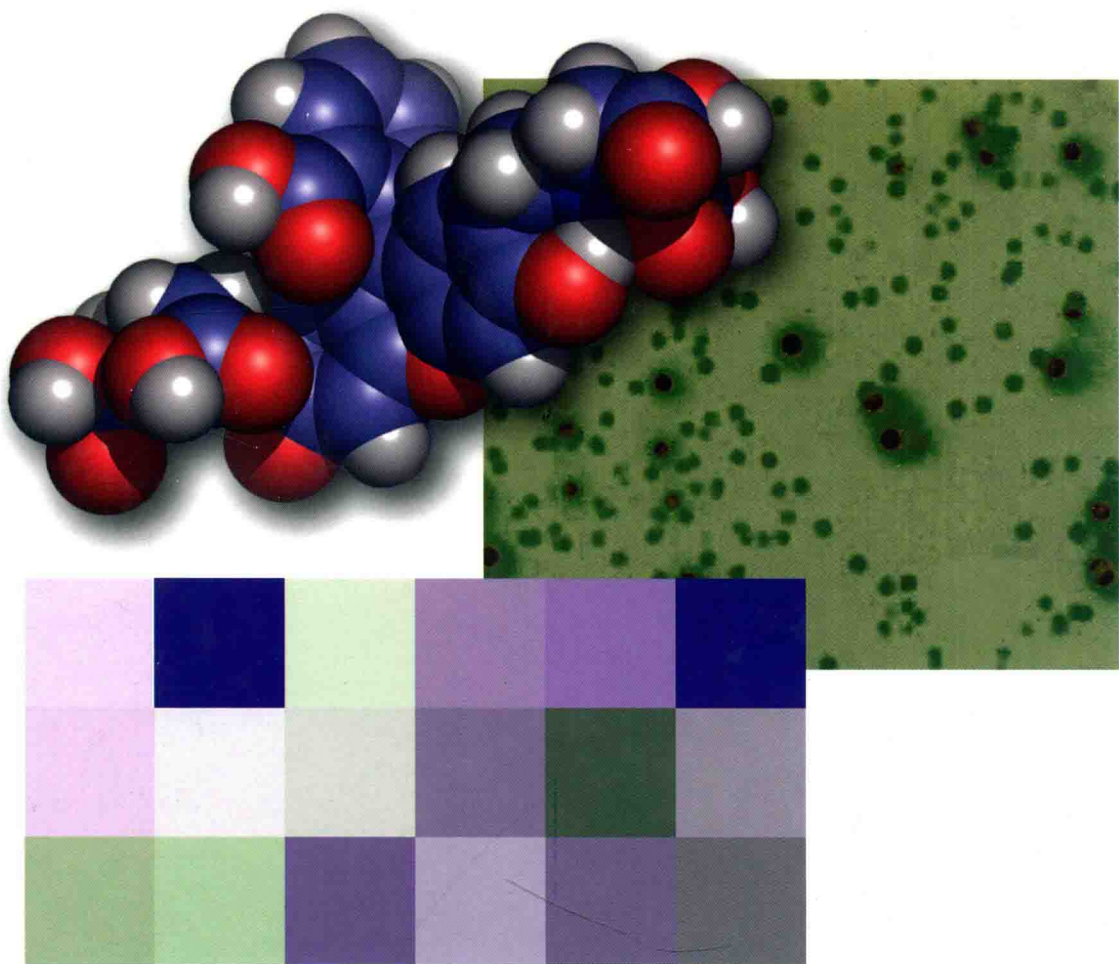


Edited by Jean-Louis Reymond

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Enzyme Assays

High-throughput Screening,
Genetic Selection and Fingerprinting



Enzyme Assays

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Genetic Selection and Fingerprinting

Edited by
Jean-Louis Reymond



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Cover illustration

The cover picture shows a cpk model of calcein (upper left), a fluorescent sensor useful for high-throughput screening of acylases, aminopeptidases, and proteases, as discussed in the Introduction.

The image on the right is a close-up view of an agar plate with colonies expressing mutant monoamine oxidases in the presence of (*S*)- α -methyl benzylamine as substrate and 3,3'-diaminobenzidine as sensor. Colony staining results from chemical oxidation of 3,3'-diaminobenzidine by the hydrogen peroxide produced in the enzyme oxidation, as discussed in Chapter 5.

The bottom grid shows a fingerprint of activity (color intensity) and enantioselectivity (purple = *R*-enantioselectivity, green = *S*-enantioselectivity) of *Bacillus thermocatenulatus* lipase (BTL2) on chiral ester substrates using the assay described in Chapter 1 and the color coding method in Chapter 10. The cover was based on a prototype by Peter Bernhardt.

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Preface

When I discuss an enzyme assay with a chemist, we spend our time devising a process that will turn an enzymatic reaction into a detectable signal. The challenge lies in the synthesis of the molecular elements involved in the assay and whether they will behave as expected. Enzyme assay design has elements of rational drug design if it requires docking an unnatural substrate into an enzyme's active site. An enzyme assay may also offer a testbed for a supramolecular functional device, serving to demonstrate its utility. Eventually new principles emerge that might change enzyme analytics altogether.

Then I turn to the biochemist or microbiologist, who sees the enzyme assay as one of many elements in a broader setup, such as the genetic selection of an active enzyme, or the study of its function and mechanism. We usually settle for a commercially available probe or couple the enzyme reaction to a biological system. Our attention focuses on the genetic design of the experiment or its biochemical interpretation. When it succeeds, we wonder with amazement at the results which we only very partly understand.

Finally I meet the industrial researcher, who is hard pressed for preparative performance within a short time window. Our discussion is narrowed down by tight specifications bound to the goals and methods. Nevertheless, the unaltered passion of the scientist keeps shining through. In addition, the products of industrial research and development are remarkable and vindicate the efforts of the entire community.

Romas Kazlauskas, Manfred Reetz, Huimin Zhao, Theo Sonke, Nick Turner, Dan Tawfik, Andrew Griffiths, Virginia Cornish, Valéria Maia de Oliveira, Gilson Paulo Manfio, Albin Hermetter, Jennifer Harris, and Yao Qin Shao have agreed to join forces with me to compose a book on enzyme assays. These authors belong to the world's leading figures in this area. I thank them and their co-authors for their time and efforts, which were essential to the project. I also thank my co-authors and students Johann Grognum and Renaud Sicard, and Elke Maase and Romy Kirsten at Wiley-VCH, for their precious help in editing.

The field of enzyme assays is evolving rapidly and touches an ever increasing number of applications. The present volume captures what we as authors believe is a fair coverage of the area at that point in time. We hope that the book will prove a useful source of information, inspiration, and references for its readers across chemistry and biology.

Berne, October 2005

Jean-Louis Reymond

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Contents

Preface XIII

List of Contributors XV

Introduction 1

Renaud Sicard and Jean-Louis Reymond

Enzyme Assays 1

Part I: The Chemistry of Enzyme Assays 7

Part II: Enzyme Assays and Genetic Selection 7

Part III: Enzyme Profiling 9

Enzyme Assays in Other Areas 11

How to Use this Book 11

Part I High-throughput Screening 15

1 Quantitative Assay of Hydrolases for Activity and Selectivity Using Color Changes 17

Romas J. Kazlauskas

1.1 Overview 17

1.2 Direct Assays Using Chromogenic Substrates 18

1.3 Indirect Assays Using Coupled Reactions – pH Indicators 19

1.3.1 Overview of Quantitative Use of pH Indicator Assay 21

1.3.2 Applications 24

1.3.2.1 Searching for an Active Hydrolase
(Testing Many Hydrolases Toward One Substrate) 24

1.3.2.2 Substrate Mapping of New Hydrolases
(Testing Many Substrates Toward Hydrolase) 25

1.3.3 Comparison with Other Methods 26

1.4 Estimating and Measuring Selectivity 27

1.4.1 Estimating Selectivity without a Reference Compound 28

1.4.2 Quantitative Measure of Selectivity Using a Reference Compound
(Quick *E* and Related Methods) 30

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- 1.4.2.1 Chromogenic Substrate 32
- 1.4.2.2 pH Indicators 33
- 1.4.3 Application 33
- 1.4.3.1 Substrate Mapping of Hydrolases 33
- 1.4.3.2 Screening of Mutants in Directed Evolution 33
- 1.4.4 Advantages and Disadvantages 36
- References* 38

2 High-throughput Screening Systems for Assaying the Enantioselectivity of Enzymes 41

Manfred T. Reetz

- 2.1 Introduction 41
- 2.2 UV/Vis Spectroscopy-based Assays 42
- 2.2.1 Assay for Screening Lipases or Esterases in the Kinetic Resolution of Chiral *p*-Nitrophenyl Esters 43
- 2.2.2 Enzyme-coupled UV/Vis-based Assay for Lipases and Esterases 45
- 2.2.3 Enzymatic Method for Determining Enantiomeric Excess (EMDee) 46
- 2.2.4 UV/Vis-based Enzyme Immunoassay as a Means to Measure Enantiomeric Excess 47
- 2.2.5 Other UV/Vis-based *ee*-Assays 48
- 2.3 Assays Using Fluorescence 48
- 2.3.1 Umbelliferone-based Systems 48
- 2.3.2 Fluorescence-based Assay Using DNA Microarrays 51
- 2.3.3 Other Fluorescence-based *ee*-Assays 53
- 2.4 Assays Based on Mass Spectrometry (MS) 53
- 2.4.1 MS-based Assay Using Isotope Labeling 53
- 2.5 Assays Based on Nuclear Magnetic Resonance Spectroscopy 58
- 2.6 Assay Based on Fourier Transform Infrared Spectroscopy for Assaying Lipases or Esterases 62
- 2.7 Assays Based on Gas Chromatography 65
- 2.8 Assays Based on HPLC 68
- 2.9 Assays Based on Capillary Array Electrophoresis 69
- 2.10 Assays Based on Circular Dichroism (CD) 71
- 2.11 Assay Based on Surface-enhanced Resonance Raman Scattering 73
- 2.12 Conclusions 73
- References* 74

3 High-throughput Screening Methods Developed for Oxidoreductases 77

Tyler W. Johannes, Ryan D. Woodyer, and Huimin Zhao

- 3.1 Introduction 77
- 3.2 High-throughput Methods for Various Oxidoreductases 78
- 3.2.1 Dehydrogenases 78

3.2.1.1	Colorimetric Screen Based on NAD(P)H Generation	78
3.2.1.2	Screens Based on NAD(P)H Depletion	79
3.2.2	Oxidases	80
3.2.2.1	Galactose Oxidase	80
3.2.2.2	D-Amino Acid Oxidase	82
3.2.2.3	Peroxidases	82
3.2.3	Oxygenases	85
3.2.3.1	Assays Based on Optical Properties of Substrates and Products	85
3.2.3.2	Assays Based on Gibbs' Reagent and 4-Aminoantipyrine	86
3.2.3.3	<i>para</i> -Nitrophenoxy Analog (pNA) Assay	87
3.2.3.4	Horseradish Peroxidase-coupled Assay	88
3.2.3.5	Indole Assay	89
3.2.4	Laccases	89
3.2.4.1	ABTS Assay	90
3.2.4.2	Poly R-478 Assay	90
3.2.4.3	Other Assays	90
3.3	Conclusions	91
	References	92
4	Industrial Perspectives on Assays	95
	<i>Theo Sonke, Lucien Duchateau, Dick Schipper, Gert-Jan Euverink, Joerd van der Wal, Huub Henderickx, Roland Bezemer, and Aad Vollebregt</i>	
4.1	Introduction	95
4.2	Prerequisites for an Effective Biocatalyst Screening in Chemical Custom Manufacturing	97
4.3	CCM Compliant Screening Methods Based on Optical Spectroscopy (UV/Vis and Fluorescence)	101
4.3.1	Optical Spectroscopic Methods Based on the Spectral Properties of the Product Itself	101
4.3.1.1	Example: Isolation of the D- <i>p</i> -Hydroxyphenylglycine Aminotransferase Gene	102
4.3.2	Optical Spectroscopic Methods Based on Follow-up Conversion of Product	104
4.3.2.1	Example: Fluorometric Detection of Amidase Activity by <i>o</i> -Phthaldehyde/Sulfite Derivatization of Ammonia	106
4.3.2.2	Example: Colorimetric Detection of Amidase Activity by Detection of Ammonia via Glutamate Dehydrogenase-coupled Assay	108
4.3.2.3	Example: Colorimetric Detection of Amino Amidase Activity Using Cu ²⁺ as Sensor for Amino Acids	112
4.4	CCM Compliant Screening Methods Based on Generic Instrumental Assays	114
4.4.1	Flow-injection NMR as Analytical Tool in High-throughput Screening for Enzymatic Activity	115

4.4.1.1	History	115
4.4.1.2	Current Practice	117
4.4.1.3	Practical Aspects	119
4.4.1.4	Example: Screening of a Bacterial Expression Library for Amidase-containing Clones	122
4.4.1.5	Example: Identification of a Phenylpyruvate Decarboxylase Clone	124
4.4.1.6	Example: Identification of Amidase Mutants with Improved Activity towards α -Methylphenylglycine Amide	125
4.4.2	Fast LC/MS for High-throughput Screening of Enzymatic Activity	126
4.4.2.1	Example: Screening of a Bacterial Expression Library for Amidase-containing Clones	127
4.4.2.2	Example: Screening of Enzymatic Racemase Activity	129
4.5	Conclusions	132
	References	133

Part II Genetic Selection 137

5 Agar Plate-based Assays 139

Nicholas J. Turner

5.1	Introduction	139
5.1.1	Directed Evolution of Enzymes: Screening or Selection?	139
5.1.2	General Features of Agar Plate-based Screens	141
5.2	Facilitated Screening-based Methods	143
5.2.1	Amidase	143
5.2.2	Esterase	144
5.2.3	Glycosynthase	144
5.2.4	Galactose Oxidase	146
5.2.5	Monoamine Oxidase	147
5.2.6	P450 Monooxygenases	150
5.2.7	Carotenoid Biosynthesis	151
5.2.8	Biotin Ligase	153
5.3	<i>In vivo</i> Selection-based Methods	154
5.3.1	Glycosynthase	154
5.3.2	Prephenate Dehydratase/Chorismate Mutase	155
5.3.3	Terpene Cyclase	157
5.3.4	Tryptophan Biosynthesis	157
5.3.5	Ribitol Dehydrogenase	157
5.3.6	Inteins	158
5.3.7	Aminoacyl-tRNA Synthetase	159
5.4	Conclusions and Future Prospects	159
	References	160

6 High-throughput Screens and Selections of Enzyme-encoding Genes 163

Amir Aharoni, Cintia Roodveldt, Andrew D. Griffiths, and Dan S. Tawfik

- 6.1 Introduction 163
- 6.2 The Basics of High-throughput Screens and Selections 164
- 6.3 High-throughput Selection of Enzymes Using Phage Display 165
- 6.4 High-throughput Selection of Enzymes Using Cell Display 168
- 6.5 *In vivo* Genetic Screens and Selections 169
- 6.6 Screens for Heterologous Protein Expression and Stability 169
 - 6.6.1 Introduction 169
 - 6.6.2 Screening Methodologies for Heterologous Expression 171
 - 6.6.3 Directed Evolution for Heterologous Expression – Recent Examples 173
- 6.7 *In vitro* Compartmentalization 174
- 6.8 IVC in Double Emulsions 177
- 6.9 Concluding Remarks 179
- References* 179

7 Chemical Complementation

Scott Lefurgy and Virginia Cornish 183

- 7.1 Introduction 183
- 7.2 Complementation Assays 184
 - 7.2.1 Introduction 184
 - 7.2.2 Early Complementation Assays 184
 - 7.2.3 Enzymology by Complementation 186
 - 7.2.4 Directed Evolution by Complementation 188
- 7.3 Development of Chemical Complementation 191
 - 7.3.1 Introduction 191
 - 7.3.2 Three-hybrid Assay 192
 - 7.3.2.1 Original Yeast Three-hybrid System 192
 - 7.3.2.2 Dexamethasone–Methotrexate Yeast Three-hybrid System 194
 - 7.3.2.3 Technical Considerations 196
 - 7.3.2.4 Other Three-hybrid Systems 198
 - 7.3.3 Chemical Complementation 198
 - 7.3.3.1 Selection Scheme and Model Reaction 199
 - 7.3.3.2 Results 202
 - 7.3.3.3 General Considerations 203
 - 7.3.3.4 Related Methods 203
- 7.4 Applications of Chemical Complementation 204
 - 7.4.1 Introduction 204
 - 7.4.2 Enzyme–Inhibitor Interactions 204
 - 7.4.2.1 Rationale 205
 - 7.4.2.2 Screen Strategy 205

7.4.2.3	Enzyme Library Screen	208
7.4.2.4	General Considerations	210
7.4.3	Glycosynthase Evolution	210
7.4.3.1	Rationale	211
7.4.3.2	Selection Scheme	212
7.4.3.3	Glycosynthase Assay	213
7.4.3.4	Directed Evolution	215
7.4.3.5	General Considerations	216
7.5	Conclusion	216
	<i>References</i>	217

8 Molecular Approaches for the Screening of Novel Enzymes

Valéria Maia de Oliveira and Gilson Paulo Manfio 221

8.1	Introduction	221
8.2	Use of Nucleic Acid Probes to Detect Enzyme-coding Genes in Cultivated Microorganisms	222
8.2.1	Current Knowledge and Applications	223
8.2.2	Limitations of Probe Technology and the Need for Innovative Approaches	224
8.3	The Microbial Metagenome: a Resource of Novel Natural Products and Enzymes	226
8.3.1	Accessing the Uncultivated Biodiversity: the Community DNA Concept	226
8.3.2	Unravelling Metabolic Function: the BAC Strategy	227
8.3.3	Analysis of Metagenomic Libraries: Activity versus Sequence-driven Strategy, Enrichment for Specific Genomes and Application of High-throughput Screening Methods	230
8.3.4	Follow-up of the Metagenome Harvest	233
8.4	Concluding Remarks	235
	<i>References</i>	236

Part III Enzyme Fingerprinting 239

9 Fluorescent Probes for Lipolytic Enzymes 241

Ruth Birner-Grünberger, Hannes Schmidinger, Alice Loidl, Hubert Scholze, and Albin Hermetter

9.1	Introduction	241
9.2	Fluorogenic and Fluorescent Substrates for Enzyme Activity	242
9.2.1	Triacylglycerol Lipase Activity Assay	245
9.2.2	Diacylglycerol Lipase Activity Assay	247
9.2.3	Cholesteryl Esterase Activity Assay	249
9.2.4	Phospholipase Activity Assay	250
9.2.5	Sphingomyelinase Activity Assay	252
9.3	Fluorescent Inhibitors for Quantitative Analysis of Active Enzymes and Functional Enzyme Fingerprinting	254

9.3.1	Lipase and Esterase Profiling	254
9.3.1.1	Microbial Lipases and Esterases	255
9.3.1.2	Porcine Pancreatic Lipase	257
9.3.1.3	Hormone-sensitive Lipase	257
9.3.2	Probing Biophysical Enzyme Properties	259
9.3.3	Affinity-based Proteome Profiling (ABPP)	262
9.3.3.1	Functionality-based Serine Hydrolase Profiling in Tissue Preparations and Cell Lines	263
	<i>References</i>	267

10 Fingerprinting Methods for Hydrolases

Johann Grognux and Jean-Louis Reymond 271

10.1	Introduction	271
10.1.1	One Enzyme – One Substrate	273
10.1.2	Enzyme Activity Profiles	275
10.1.3	The APIZYM System for Microbial Strain Identification	276
10.2	Hydrolase Fingerprinting	278
10.2.1	Fingerprinting with Fluorogenic and Chromogenic Substrates	279
10.2.2	Fingerprinting with Indirect Chromogenic Assays	284
10.2.3	Cocktail Fingerprinting	287
10.3	Classification from Fingerprinting Data	289
10.3.1	Fingerprint Representation	290
10.3.2	Data Normalization	293
10.3.3	Hierarchical Clustering of Enzyme Fingerprints	295
10.3.4	Analysis of Substrate Similarities	297
10.4	Outlook	299
	<i>References</i>	300

11 Protease Substrate Profiling

Jennifer L. Harris 303

11.1	Introduction	303
11.2	Functional Protease Profiling – Peptide Substrate Libraries	304
11.2.1	Solution-based Peptide Substrate Libraries	306
11.2.2	Solid Support-based Synthesis and Screening of Peptide Libraries	314
11.2.3	Genetic Approaches to Identifying Protease Substrate Specificity	320
11.3	Identification of Macromolecular Substrates	322
11.3.1	Genetic Approach to the Identification of Macromolecular Substrates	323
11.3.2	Proteomic Approaches to Identifying Protease Substrates	326
11.4	Conclusions	327
	<i>References</i>	328