Edited by Jean-Louis Reymond

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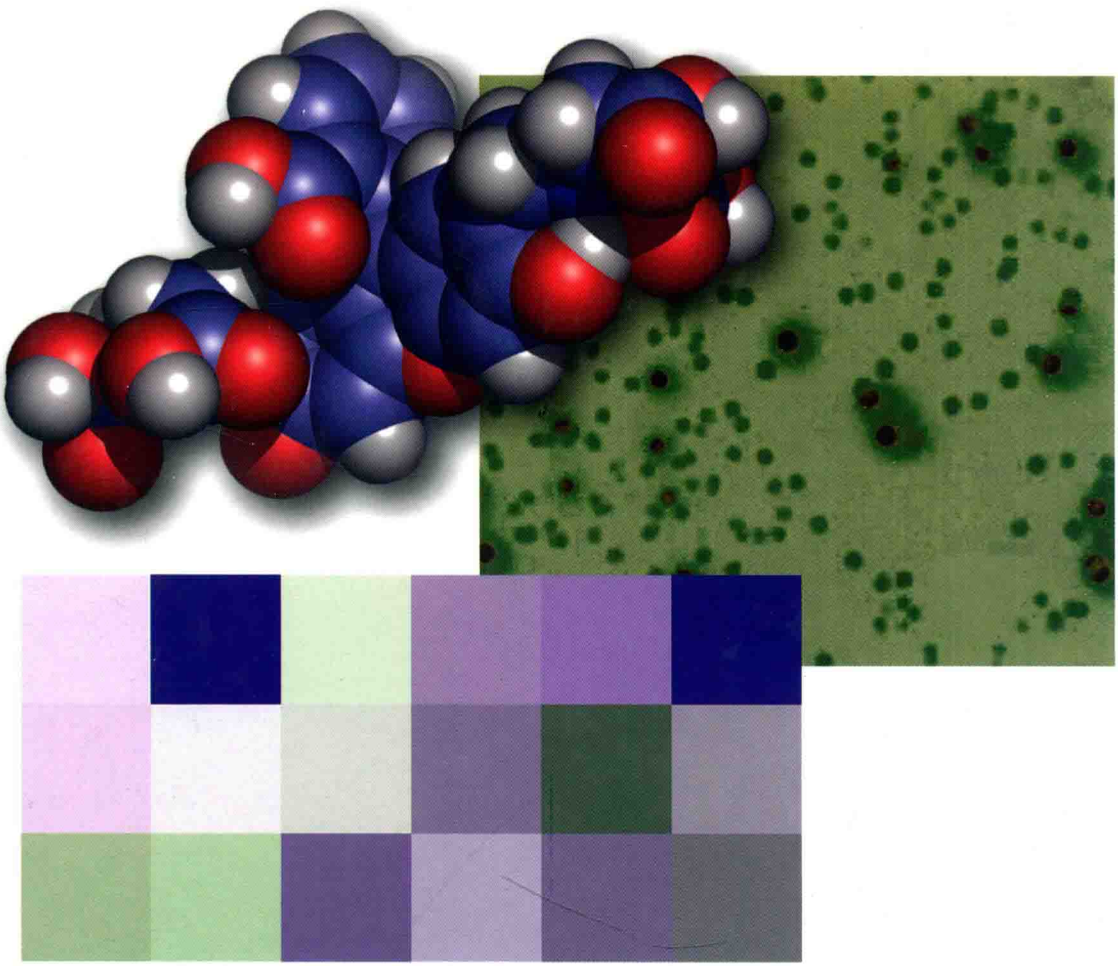
Enzyme Assays

High-throughput Screening, Genetic Selection and Fingerprinting



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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*)-alpha-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 Grognux 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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