

Pharmaceutical Biotechnology

Drug Discovery and Clinical Applications

Second, Completely Revised,
and Greatly Enlarged Edition

Edited by Oliver Kayser
and Heribert Warzecha



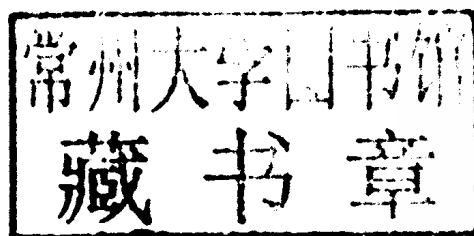
WILEY-
BLACKWELL

Edited by Oliver Kayser and Heribert Warzecha

Pharmaceutical Biotechnology

Drug Discovery and Clinical Applications

Second, Completely Revised, and Greatly Enlarged Edition



The Editors

Prof. Dr. Oliver Kayser

Technical University Dortmund
Laboratory of Technical
Biochemistry
Emil-Figge-Straße 68
44227 Dortmund
Germany

Prof. Dr. Heribert Warzecha

TU Darmstadt
Biological Science
Schnittspahnstraße 3
64287 Darmstadt
Germany

Cover

250 L Setup, © Rentschler
Biotechnologie GmbH

DNA molecule

© mauritius images/Science
Photos Library

Limit of Liability/Disclaimer of Warranty: While the publisher and author have used their best efforts in preparing this book, they make no representations or warranties with respect to the accuracy or completeness of the contents of this book and specifically disclaim any implied warranties of merchantability or fitness for a particular purpose. No warranty can be created or extended by sales representatives or written sales materials. The Advice and strategies contained herein may not be suitable for your situation. You should consult with a professional where appropriate. Neither the publisher nor authors shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library.

Bibliographic information published by the Deutsche Nationalbibliothek

The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at <<http://dnb.d-nb.de>>.

© 2012 Wiley-VCH Verlag & Co. KGaA,
Boschstr. 12, 69469 Weinheim, Germany

Wiley-Blackwell is an imprint of John Wiley & Sons, formed by the merger of Wiley's global Scientific, Technical, and Medical business with Blackwell Publishing.

All rights reserved (including those of translation into other languages). No part of this book may be reproduced in any form – by photoprinting, microfilm, or any other means – nor transmitted or translated into a machine language without written permission from the publishers. Registered names, trademarks, etc. used in this book, even when not specifically marked as such, are not to be considered unprotected by law.

Print ISBN: 978-3-527-32994-6

ePDF ISBN: 978-3-527-65126-9

ePub ISBN: 978-3-527-65125-2

mobi ISBN: 978-3-527-65124-5

oBook ISBN: 978-3-527-63290-9

Composition Toppan Best-set Premedia Limited,
Hong Kong

Printing and Binding betz-druck GmbH,
Darmstadt

Cover Design Adam-Design, Weinheim

Printed in the Federal Republic of Germany
Printed on acid-free paper

Edited by
Oliver Kayser and
Heribert Warzecha

Pharmaceutical Biotechnology

Related Titles

Behme, S.

Manufacturing of Pharmaceutical Proteins

From Technology to Economy

2009

ISBN: 978-3-527-32444-6

Walsh, G.

Pharmaceutical Biotechnology

Concepts and Applications

2007

ISBN: 978-0-470-01245-1

Walsh, G. (ed.)

Post-translational Modification of Protein Biopharmaceuticals

2009

ISBN: 978-3-527-32074-5

Gad, S. C. (ed.)

Handbook of Pharmaceutical Biotechnology

2007

ISBN: 978-0-471-21386-4

Tobin, J. J., Walsh, G.

Medical Product Regulatory Affairs

**Pharmaceuticals, Diagnostics, Medical
Devices**

2008

ISBN: 978-3-527-31877-3

Preface to the 2nd Edition

Pharmaceutical biotechnology has emerged as one of the major disciplines for drug discovery and development. In the past, the pharmaceutical branch of biotechnology – the former red biotechnology – was limited to fermentation and production of recombinant therapeutic proteins. Today, the shape and vision of pharmaceutical aspects and challenges have completely changed, and the prefix “pharma” can also be accepted as a synonym for integrated life science approaches, ranging from genetics to molecular biology to diagnostics, with the common goal of delivering the best drug to the patient by biotechnological techniques.

If we take a look at the first edition of *Pharmaceutical Biotechnology*, we see that the focus was more on molecules as potential drugs and less on the production strategies and the molecular concepts behind. The completely updated and rewritten second edition reflects the emerging trend in the pharmaceutical industry where molecular biology techniques and genetics play an increasingly important role. Today, many new biological entities can be characterized as muteins or significantly backbone-modified proteins, an exception in 2004 when we published the first edition (see insulin muteins). We are glad that we were able to attract the majority of the authors from the previous edition as experts. They reviewed the latest trends in their subjects of expertise and shared their experience and open opinion about the developments from the recent years to the near future. Pharmaceutical biotechnology and the pharmaceutical industry is a fast moving business and we all know that the future is hard to predict, but we are glad that with the selected contributors being in touch with industrial needs and challenges, we made the right choice to give answers to the readers’ questions not only about new developments in protein production, host organism selection, and future platform organisms for biosynthesis and vaccine production, but also on biological generics, drug formulation, and legal aspects of biotechnology. In this textbook you will find updated facts and figures about the pharmaceutical industry and the latest drug approvals. In the first part a detailed discussion is provided about production systems for the biosynthesis of both low molecular weight drugs and proteins in prokaryotic and eukaryotic cell cultures and organisms. In the second part the drug formulation and manufacturing process is in focus, but we also want to highlight quality control and bioanalytical aspects, which have been largely neglected before. Therefore, this second part is now updated and dedicated to the

recombinant therapeutic proteins and vaccines that are already in clinical use, as well as requirements for quality control. In contrast to the first edition we recognized that drug regulation and quality assurance are becoming more important, while the legal aspects of drug patenting, and the drug approval process are again emphasized. In the third part we had a hard task of sorting and structuring the emerging diversity of research and development in this field and bring it under one single chapter. This is nearly impossible, but our aim is to guide the reader through the new upcoming lines of research impacted by genetics, synthetic biology, and nanobiotechnology. Finally we selected chapters showing exemplarily ongoing research trends that, hopefully, will find their way into clinical applications in the future or as approved drugs into the second edition of this textbook. Well-updated by authors from the previous edition, we learn about personalized medicine and xenotransplantation, and we are proud to introduce new contributors telling us about nanocarriers as future drug delivery systems, ultrahigh-throughput screening for accelerated drug discovery, and transgenic plants as future green factories.

The editors want to thank all the authors for their valuable contributions and the time they have invested in this work. We know very well that time was and is a scarce resource and that the chapters were written alongside the authors' regular duties. Special thanks also to the families behind for their patience and understanding why time was spent in this project. Special thanks to Anne Chassin du Guerny and Gregor Cichetti of Wiley-Blackwell for their professional support in the layout, proofreading, and production of this textbook.

We know that this book is far from being complete and we are aware that by the day of publishing it could be updated again. But our intention is to provide a "primer" for the interested reader to start working and to show how exciting research is in this fast moving field of life science.

Dortmund and Darmstadt, January 2012

*Oliver Kayser
Heribert Warzecha*

List of Contributors

Shoaib Ahmad

Rayat and Bahra Institute of Pharmacy
Department of Pharmacology
Sahauran, Punjab 140104
India

Michael Balls

Fund for the Replacement of Animals
in Medical Experiments (FRAME)
Russell & Burch House
96–98 North Sherwood Street
Nottingham NG1 4EE
UK

Debmalya Barh

Institute of Integrative Omics and
Applied Biotechnology (IIOAB)
PB Barh Centre for Bioprocess,
Biotechnology, and Renewable Energy
Nonakuri, Purba Medinipur
West Bengal 721172
India

Ross T. Barnard

The University of Queensland,
St Lucia
Australian Infectious Diseases
Research Centre
School of Chemistry and Molecular
Biosciences
Brisbane, Queensland 4072
Australia

Andreas Bechthold

Albert Ludwigs University of Freiburg
Department of Pharmaceutical Biology
and Biotechnology
Institute for Pharmaceutical Sciences
79104 Freiburg
Germany

Andrew Bennett

University of Nottingham Medical
School
School of Biomedical Sciences
FRAME Alternatives Laboratory
Queen's Medical Centre
Nottingham NG7 2UH
UK

Atanu Bhattacharjee

North Eastern Hill University
Department of Biotechnology and
Bioinformatics
Shillong 22
India

Gregory J. Brunn

Mayo Clinic
Department of Molecular
Pharmacology and Experimental
Therapeutics
Rochester, MN 55905
USA

Maria J. De Jesus

ExcellGene SA
Route de l'île-au-bois 1A
1870 Monthey
Switzerland

Theo Dingermann

Goethe University Frankfurt
Institute of Pharmaceutical Biology
Max-von-Laue-Straße 9
60438 Frankfurt/Main
Germany

Sean M. Geary

University of Iowa
Department of Pharmaceutical
Sciences and Experimental
Therapeutics
College of Pharmacy
M5S. Grand Avenue
Iowa City, IA 52242
USA

Christoph Giese

ProBioGen AG
Goethestraße 54
13086 Berlin
Germany

Uwe Gottschalk

Sartorius-Stedim Biotech
Purification Technologies
August-Spindler-Straße 11
37079 Göttingen
Germany

Jens-Peter Gregersen

Novartis Vaccines and
Diagnostics GmbH
Emil-von-Behring Straße 76
35041 Marburg
Germany

Nizar Happyana

Technical University of Dortmund
Laboratory of Technical Biochemistry
Emil-Figge Straße 66
44227 Dortmund
Germany
and
Bandung Institute of Technology
Department of Chemistry
Jl. Ganesha 10
Bandung 40132
Indonesia

Oktavia Hendrawati

University of Groningen
Pharmaceutical Biology Department
Antonius Deusinglaan 1
9713 AV Groningen
The Netherlands

Jacques Hille

University of Groningen
Molecular Biology of Plants
Department
Kerklaan 30
9751 NN Haren
The Netherlands

Walter Hinderer

Gedeon Richter Pharma GmbH
Robert-Bosch-Straße 11B
63225 Langen
Germany

Henning von Horsten

ProBioGen AG
Goethestraße 54
13086 Berlin
Germany

Kewal K. Jain

Jain PharmaBiotech
Blaesiring 7
4057 Basel
Switzerland

Oliver Kayser

Technical University Dortmund
Laboratory of Technical Biochemistry
Emil-Figge Straße 66
44227 Dortmund
Germany

David Kendall

University of Nottingham
Medical School
School of Biomedical Sciences
FRAME Alternatives Laboratory
Queen's Medical Centre
Nottingham NG7 2UH
UK

Alexander Kind

Technical University Munich
Department of Livestock
Biotechnology
Liesel-Beckmann Straße 1
85354 Freising-Weihenstephan
Germany

Yogita Krishnamachari

University of Iowa
Department of Pharmaceutical
Sciences and Experimental
Therapeutics
College of Pharmacy
M5S. Grand Avenue
Iowa City, IA 52242
USA

Luke R. Le Grand

The University of Queensland,
St. Lucia
School of Chemistry and Molecular
Biosciences
Brisbane, Queensland 4072
Australia

Caitlin D. Lemke

University of Iowa
Department of Pharmaceutical
Sciences and Experimental
Therapeutics
College of Pharmacy
M5S. Grand Avenue
Iowa City, IA 52242
USA

Andriy Luzhetskyy

Albert Ludwigs University of Freiburg
Department of Pharmaceutical Biology
and Biotechnology
Institute for Pharmaceutical Sciences
79104 Freiburg
Germany

Bernd Meibohm

University of Tennessee Health
Science Center
College of Pharmacy
Department of Pharmaceutical
Sciences
Memphis, TN 38163
USA

Remco Muntendam

University of Groningen
Department of Pharmaceutical Biology
Antonius Deusinglaan 1
9713 AV Groningen
The Netherlands

Julia Myschik

Ludwig-Maximilians-University
Munich
Department of Pharmacy
Pharmaceutical Technology and
Biopharmaceutics
Butenandtstraße 5
81377 Munich
Germany

Heiner Niemann

Friedrich Loeffler Institute (FLI)
Institute of Farm Animal Genetics
Mariensee
31535 Neustadt
Germany

Jeffrey L. Platt

University of Michigan
Departments of Surgery and
Microbiology & Immunology
Ann Arbor, MI 48109
USA

David B. Resnik

East Carolina University
The Brody School of Medicine
Greenville, NC 27858
USA

Aliasger K. Salem

University of Iowa
Department of Pharmaceutical
Sciences and Experimental
Therapeutics
College of Pharmacy
M5S. Grand Avenue
Iowa City, IA 52242
USA

Angelika Schnieke

Technical University Munich
Department of Livestock
Biotechnology
Liesel-Beckmann Straße 1
85354 Freising-Weihenstephan
Germany

Evan B. Siegel

Ground Zero Pharmaceuticals
2600 Michelson Drive
Irvine, CA 92612
USA

Gary Walsh

University of Limerick
Industrial Biochemistry Program and
the Materials and Surface Science
Institute
Limerick City
Ireland

Heribert Warzecha

Technische Universität Darmstadt
Biology
Schnittspahnstraße 3-5
64267 Darmstadt
Germany

Gabriele Weitnauer

Albert Ludwigs University of Freiburg
Department of Pharmaceutical Biology
and Biotechnology
Institute for Pharmaceutical Sciences
79104 Freiburg
Germany

Michaela White

Thomson Reuters
Life Sciences
Asia Pacific
Melbourne, Victoria 3001
Australia

Gerhard Winter

Ludwig-Maximilians-University
Munich
Department of Pharmacy
Pharmaceutical Technology and
Biopharmaceutics
Butenandtstraße 5
81377 Munich
Germany

Herman J. Woerdenbag

University of Groningen
Pharmaceutical Technology and
Biopharmacy Department
Antonius Deusinglaan 1
9713 AV Groningen
The Netherlands

Florian M. Wurm

Ecole Polytechnique Fédérale de
Lausanne (EPFL)
Laboratory of Cellular Biotechnology
1015 Lausanne
Switzerland
and
ExcellGene SA
Route de l'île-au-bois 1A
1870 Monthey
Switzerland

Yi Zhang

University of Tennessee
Health Science Center
College of Pharmacy
Department of Pharmaceutical
Sciences
Memphis, TN 38163
USA

Jian Zhao

The Samuel Roberts Noble
Foundation
2510 Sam Noble Parkway
Ardmore, OK 73401
USA

Stefan Zietze

ProBioGen AG
Goethestraße 54
13086 Berlin
Germany

Ilse Zündorf

Goethe University Frankfurt
Institute of Pharmaceutical Biology
Max-von-Laue-Straße 9
60438 Frankfurt/Main
Germany

Contents

Preface *XXI*

List of Contributors *XXIII*

Part One Concepts and Methods for Recombinant Drug Production 1

- 1 **Pharmaceutical Biotechnology and Industrial Applications – Learning Lessons from Molecular Biology** 3
Oliver Kayser and Heribert Warzecha
 - 1.1 Introduction 3
 - 1.2 Research Developments 5
 - 1.2.1 Protein Engineering 5
 - 1.2.2 Muteins 6
 - 1.2.3 Post-translational Engineering 7
 - 1.2.4 Synthetic Biology 9
 - 1.3 Production Hosts and Upstream/Downstream Processing 10
 - 1.4 Future Outlook 11
 - References 12
 - Weblinks 13
- 2 **Prokaryotic Cells in Biotech Production** 15
Andriy Luzhetskyy, Gabriele Weitnauer, and Andreas Bechthold
 - 2.1 Introduction 15
 - 2.2 Production of Natural Products by Microorganisms 15
 - 2.2.1 Production of Libraries of Natural Products 16
 - 2.2.2 Production of Natural Products by Cloning and Expression of Biosynthetic Gene Clusters 18
 - 2.2.3 Culture Manipulation to Wake Up Silent Gene Clusters 19
 - 2.2.4 Genomic Driven Approaches to Wake Up Silent Gene Clusters 19
 - 2.2.5 *E. coli*, an Interesting Host Also for Natural Product Synthesis 19
 - 2.2.5.1 Production of Polyketides in *E. coli* 19
 - 2.2.5.2 Metabolic Engineering of *E. coli* for Isoprenoid Biosynthesis 20

2.2.6	Global-Scale Strategies for Strains Improvement	21
2.2.6.1	System Biology, System Biotechnology, and “Omic” Approaches	21
2.2.6.2	Synthetic Biology Tools	22
2.2.6.3	Whole Genome Engineering Approaches	24
2.3	Prokaryotes as Producers of Recombinant Therapeutic Proteins	26
2.3.1	Prokaryotic Expression Systems	27
2.3.1.1	Host Strains	27
2.3.1.2	Expression Vectors	31
2.3.2	Production Steps	34
2.3.3	Products	34
2.3.3.1	Somatropin (Somatotropin, STH, Human Growth Hormone, hGH)	34
2.3.3.2	Human Insulin	36
	References	37
3	Mammalian Cells in Biotech Production	43
	<i>Maria J. De Jesus and Florian M. Wurm</i>	
3.1	Introduction	43
3.2	Process Concepts and Cells	44
3.3	CHO-Derived Production Cell Lines	46
3.4	Rapid Generation of High-Producing Cell Lines	47
3.5	Silencing–Stability of Expression	49
3.6	High-Throughput Bioprocess Development	50
3.7	Disposable Bioreactors	51
3.8	Transient Gene Expression (TGE)	52
3.9	Conclusions	53
	References	54
4	Biopharmaceuticals from Plants	59
	<i>Heribert Warzecha</i>	
4.1	Introduction	59
4.2	Basics in Plant Biotechnology	60
4.3	Plant Cell Cultures as Production System for Human Glucocerebrosidase	63
4.4	Insulin from Safflower–A Unique Purification Scheme	64
4.5	Fast and Scalable Transient Tobacco-Based Expression Systems	65
4.6	Conclusion	67
	References	68
5	Production of Biopharmaceuticals in Transgenic Animals	71
	<i>Heiner Niemann, Alexander Kind, and Angelika Schnieke</i>	
5.1	Introduction	71
5.2	Sites of Production	73
5.2.1	Milk	73

5.2.2	Urine	76
5.2.3	Seminal Fluid	77
5.2.4	Blood	77
5.2.5	Bird Eggs	78
5.3	Transgenic Constructs	78
5.3.1	Organ Specific Expression Vectors	80
5.3.2	Inducible Expression	81
5.3.3	Non-integrating Vectors	81
5.4	Methods for the Production of Transgenic Animals	82
5.4.1	Pronuclear DNA Microinjection	83
5.4.1.1	Collection of Fertilized Eggs	83
5.4.1.2	Preparation of DNA	83
5.4.1.3	Injection of DNA	83
5.4.1.4	Transfer and Gestation in Recipients	84
5.4.1.5	Identification of Founders and Subsequent Breeding	85
5.4.2	Viral Mediated Gene Transfer	86
5.4.3	Sperm-Mediated Gene Transfer	88
5.4.4	Transposon-Mediated Gene Transfer	89
5.4.5	Pluripotent Stem Cells	90
5.4.5.1	Embryonic Stem Cells	90
5.4.5.2	Embryonic Germ Cells	91
5.4.5.3	Induced Pluripotent Stem Cells (iPS Cells)	92
5.4.6	Spermatogonial Stem Cells	93
5.4.7	Somatic Cell Nuclear Transfer	94
5.4.8	Highly Specific DNA Endonucleases	99
5.5	Analysis of Transgenic Animals	99
5.5.1	Analysis of Integrated Transgenes	100
5.5.2	Transgene-Expression Profile	101
5.5.3	Collection, Processing, and Protein Purification	101
5.6	Quality and Safety of the Product	102
5.7	Conclusions and Outlook	104
	References	105
6	Translation of New Technologies in Biomedicines: Shaping the Road from Basic Research to Drug Development and Clinical Application—and Back Again	113
	<i>Michael Balls, Andrew Bennett, and David Kendall</i>	
6.1	Drug Discovery and Development	113
6.2	The Nature of Models and the Need for Them	114
6.3	New Technologies Toolbox	116
6.3.1	Use of Existing Knowledge	117
6.3.2	<i>In Chemico</i> and Other Physicochemical Approaches	118
6.3.3	<i>In Silico</i> Methods	119
6.3.3.1	<i>In Silico</i> Methods and Drug Discovery	120
6.3.3.2	<i>In Silico</i> Methods and Toxicology	121

6.3.4	<i>In Vitro</i> Systems	122
6.3.4.1	Cell Fractions	122
6.3.4.2	Cell Monolayer or Suspension Cultures	123
6.3.4.3	Co-cultures, Organotypic Cultures, and Reconstituted Tissue Constructs	124
6.3.4.4	Tissue Engineering	125
6.3.4.5	Stem Cells	125
6.3.4.6	Examples of Some Specific <i>In Vitro</i> Systems	127
6.3.4.7	Dynamic Bioreactors	127
6.3.4.8	Multi-organ Systems	128
6.3.4.9	Challenge of Cells, Organs, and Organisms on a Chip	129
6.3.4.10	<i>In Vitro</i> Assays	129
6.3.4.11	Coordinated Approach with <i>In Vitro</i> Models: the Vitrocellomics Project	130
6.3.5	High-Throughput Screening	131
6.3.6	High-Content Screening	131
6.3.7	Omics Approaches	131
6.3.7.1	Variety of Omics	132
6.3.7.2	Application of the Omics	132
6.3.7.3	Handling Information Produced by the Omics	133
6.3.8	Systems Modeling and Simulation	134
6.3.8.1	Pharmacokinetic Modeling	134
6.3.8.2	Virtual Tissue Modeling	135
6.3.8.3	Virtual Patient Populations	136
6.3.9	Biomarkers	136
6.3.10	Clinical Imaging	138
6.3.11	Bioinformatics	140
6.4	Strategic Use of the New Technology Tools	141
6.4.1	The Tools	142
6.4.2	The Strategies	142
6.4.3	Systems Biology	143
6.4.4	Involving the Patient	144
6.5	Translation as a Two-Way Process	145
6.6	Concluding Comment	146
	References	147

Part Two Bringing the Drug into Action—From Downstreaming to Approval 153

7	Overview and Classification of Approved Recombinant Drugs	155
	<i>Theo Dingermann and Ilse Zündorf</i>	
7.1	Introduction	155
7.2	Classification of Recombinant Drugs from a Technical Point of View	166

7.3	Expression Systems	167
7.4	Proteins Derived from Modified Genes	170
7.5	Artificial Proteins	171
7.6	Post-expression Modifications of Recombinant Proteins	173
7.7	Biosimilars	174
	References	177
8	Downstream Processing	179
	<i>Uwe Gottschalk</i>	
8.1	Introduction	179
8.2	General Principles of DSP	180
8.3	Clarification	181
8.3.1	Centrifugation	181
8.3.2	Filtration	182
8.3.3	Increasing the Efficiency of Clarification	185
8.4	Chromatography	187
8.4.1	Column Chromatography	187
8.4.2	Membrane Chromatography	188
8.4.3	Capture Chromatography	189
8.4.4	Polishing Chromatography	191
8.4.5	Continuous Chromatography	193
8.5	Ultrafiltration/Diafiltration, and Virus Filtration	194
8.5.1	Ultrafiltration/Diafiltration	194
8.5.2	Virus Filtration	195
8.6	Crystallization	196
8.7	Recent Developments in Downstream Processing	196
	References	197
9	Characterization of Recombinant Proteins	201
	<i>Christoph Giese, Henning von Horsten, and Stefan Zietze</i>	
9.1	Introduction	201
9.2	Physical Chemical Characterization	201
9.2.1	Spectroscopic Methods	201
9.2.1.1	Ultraviolet Absorption Spectroscopy	201
9.2.1.2	Fluorescence Spectroscopy	202
9.2.1.3	Fourier Transform Infrared Spectroscopy	203
9.2.2	Chromatographic Methods	205
9.2.2.1	Size-Exclusion Chromatography	205
9.2.2.2	Reversed-Phase Chromatography	206
9.2.2.3	Hydrophilic Interaction Chromatography	207
9.2.2.4	Ion-Exchange Chromatography	207
9.2.3	Electrophoretic Methods	208
9.2.3.1	Gel Electrophoresis	208
9.2.3.2	Capillary Electrophoresis	209
9.2.4	Other Physical Chemical Methods	210