

Edited by Jörg Knäblein

 WILEY-VCH

Modern Biopharmaceuticals

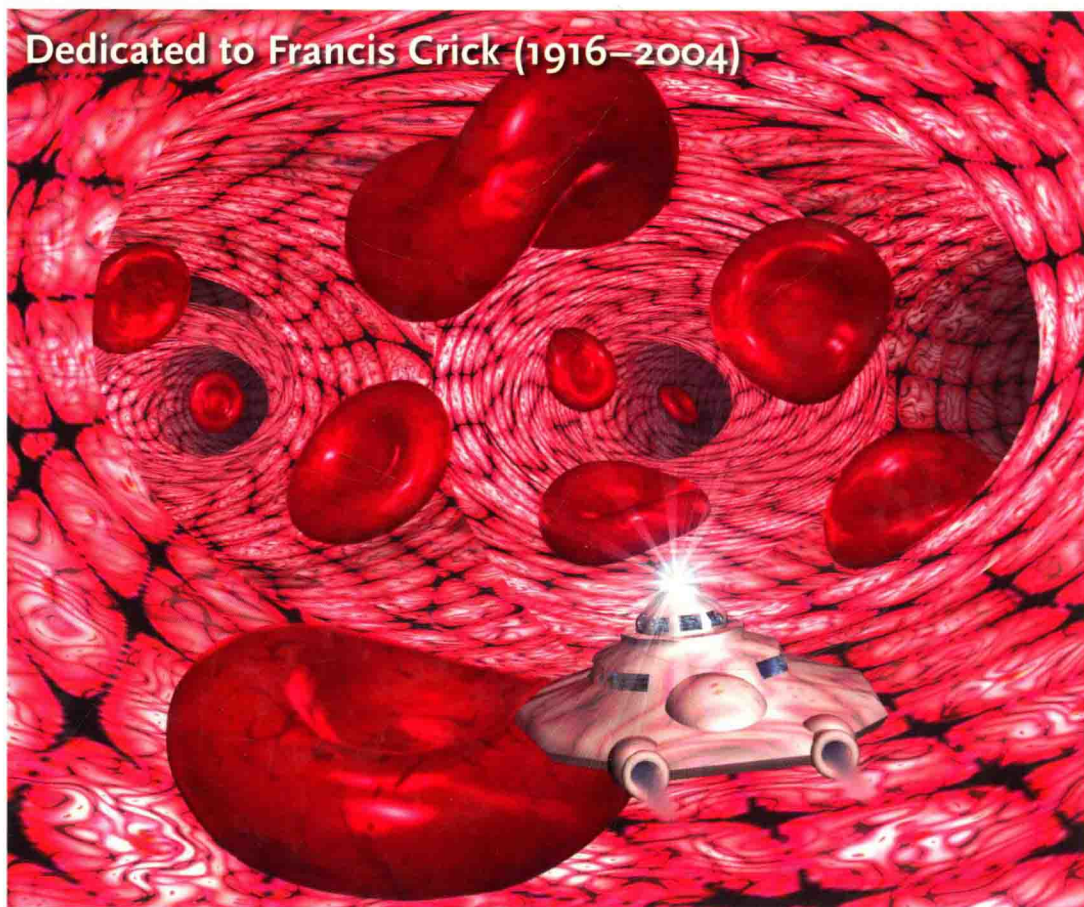
Design, Development and Optimization

Volume 3



included

Dedicated to Francis Crick (1916–2004)



Modern Biopharmaceuticals

Volume 3

Design, Development and Optimization

Edited by
Jörg Knäblein



WILEY-
VCH

WILEY-VCH Verlag GmbH & Co. KGaA

Editor

Dr. Jörg Knäblein

Head Microbiological Chemistry
Schering AG
Müllerstraße 178
13342 Berlin
Germany

All books published by Wiley-VCH are carefully produced. Nevertheless, authors, editors, and publisher do not warrant the information contained in these books, including this book, to be free of errors. Readers are advised to keep in mind that statements, data, illustrations, procedural details or other items may inadvertently be inaccurate.

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

Die Deutsche Bibliothek

Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at <http://dnb.ddb.de>.

© 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

All rights reserved (including those of translation into other languages). No part of this book may be reproduced in any form – nor transmitted or translated into 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.

Printed in the Federal Republic of Germany

Printed on acid-free paper

Cover Tim Fonseca, www.fonsecatim.com

Typesetting K+V Fotosatz GmbH, Beerfelden

Printing betz-druck GmbH, Darmstadt

Bookbinding J. Schäffer GmbH, Grünstadt

ISBN-13 978-3-527-31184-2

ISBN-10 3-527-31184-X

Jörg Knäblein (Ed.)

Modern Biopharmaceuticals

Further Titles of Interest

Gary Walsh

Biopharmaceuticals **Biochemistry and Biotechnology**

2003

ISBN 0-470-84326-8

Oliver Kayser, Rainer H. Müller (Eds.)

Pharmaceutical Biotechnology **Drug Discovery and Clinical Applications**

2004

ISBN 3-527-30554-8

Gary Walsh

Proteins **Biochemistry and Biotechnology**

2001

ISBN 0-471-89907-0

Rainer Fischer, Stefan Schillberg (Eds.)

Molecular Farming **Plant-made Pharmaceuticals and Technical Proteins**

2004

ISBN 3-527-30786-9

Rodney J.Y. Ho, Milo Gibaldi

Biotechnology **and Biopharmaceuticals** **Transforming Proteins and Genes into Drugs**

2003

ISBN 0-471-20690-3

Martin Schleef (Ed.)

DNA-Pharmaceuticals **Formulation and Delivery in Gene Therapy** **and DNA Vaccination**

2005

ISBN 3-527-31187-4

Chi-Huey Wong (Ed.)

Carbohydrate-based **Drug Discovery**

2003

ISBN 3-527-30632-3

Rolf D. Schmid, Ruth Hammelehle

Pocket Guide to Biotechnology **and Genetic Engineering**

2003

ISBN 3-527-30895-4

Contents

Volume 1

Prologue XXV

Dedication XXIX

Foreword XXXI

Foreword XXXV

Quotes XXXVII

Executive Summary XLI

List of Contributors CXXIII

Introduction

Current Status of Biopharmaceuticals: Approved Products and Trends in Approvals 1
Gary Walsh

Part I Biopharmaceuticals Used in Molecular Medicine

From Genome to Clinic – Correlation Between Genes, Diseases and Biopharmaceuticals 37

1 Beginning to Understand the End of the Chromosome 37
Thomas R. Cech

**2 The Role of Pharmacogenetics/Pharmacogenomics
in Drug Development and Regulatory Review: Current Status** 49
Shiew-Mei Huang and Lawrence J. Lesko

3	Large-scale Detection of Genetic Variation: The Key to Personalized Medicine	71
	<i>Joerg Geistlinger and Peter Ahnert</i>	
4	A Systems Biology Approach to Target Identification and Validation for Human Chronic Disease Drug Discovery	99
	<i>Bonnie E. Gould Rothberg, Carol E.A. Peña, and Jonathan M. Rothberg</i>	
5	The Development of Herceptin®: Paving the Way for Individualized Cancer Therapy	127
	<i>Thorsten S. Gutjahr and Carsten Reinhardt</i>	
	siRNA – the Magic Bullet and Other Gene Therapeutical Approaches	151
6	Adenovirus-based Gene Therapy: Therapeutic Angiogenesis with Adenovirus 5 Fibroblast Growth Factor-4 (Ad5FGF-4) in Patients with Chronic Myocardial Ischemia	151
	<i>Michael McCaman, Francisco J. Castillo, Farah Fawaz, Yasushi Ogawa, Erik Whiteley, Elisabeth Lehmborg, Mei Tan, Jacob Kung, Bruce Mann, Erno Pungor Jr., and Gabor M. Rubanyi</i>	
7	MIDGE Vectors and dSLIM Immunomodulators: DNA-based Molecules for Gene Therapeutic Strategies	183
	<i>Manuel Schmidt, Barbara Volz, and Burghardt Wittig</i>	
8	Nonprotein-coding RNAs and their Potential as Biopharmaceuticals	213
	<i>Maciej Szymanski, Jan Barciszewski and Volker A. Erdmann</i>	
9	Double-stranded Decoy Oligonucleotides as new Biopharmaceuticals	229
	<i>Andreas H. Wagner and Heiko E. von der Leyen</i>	
10	Rational siRNA Design for RNA Interference: Optimizations for Therapeutic Use and Current Applications	243
	<i>Anastasia Khvorova, Queta Boese, and William S. Marshall</i>	
	Mobilis in Mobile – Human Embryonic Stem Cells and Other Sources for Cell Therapy	269
11	The First Cloned Human Embryo: An Unlimited Source of Stem Cells for Therapeutic Cloning	269
	<i>Woo Suk Hwang, Byeong Chun Lee, Sung Keun Kang, and Shin Yong Moon</i>	
12	Myocardial Regeneration Strategies using Human Embryonic Stem Cells	283
	<i>Izhak Kehat, Oren Caspi, and Lior Gepstein</i>	
13	Gene and Cell-based Therapies for Cardiovascular Disease	305
	<i>Abeel A. Mangi</i>	

- 14 **Spheramine®: A Cell Therapeutic Approach to Parkinson's Disease** 325
Elke Reissig, Hermann Graf, and Friedrich-Joachim Kapp
- 15 **Applying Human Cells to Organogenesis and Transplantation** 353
Benjamin Dekel and Yair Reisner

Volume 2

Part II Biopharmaceuticals and Their Mode of Action

Quid pro Quo – Lysis vs. Coagulation in the Fine-tuned Balance of the Clotting Cascade 377

- 1 **Mechanisms of Serine Proteinase Activation: Insights for the Development of Biopharmaceuticals for Coagulation and Fibrinolysis** 377
Rainer Friedrich
- 2 **Application of the Principle of Polyvalency to Protease Inhibition** 395
Luis Moroder
- 3 **A New Technology Standard for Safety and Efficacy in Factor VIII Replacement Therapy: Designing an Advanced Category rFVIII Concentrate** 419
Norbert Riedel and Friedrich Dörner

Errare Humanum Est – What Causes Cancer and How to Selectively Fight Tumors 451

- 4 **Biopharmaceutical Drugs from Natural Sources** 451
David J. Newman, Gordon M. Cragg, and Barry R. O'Keefe
- 5 **Biopharmaceuticals as Targeting Vehicles for *In situ* Radiotherapy of Malignancies** 497
Raymond M. Reilly
- 6 **New Directions in Tumor Therapy – Amino Acid Depletion with GlutaDON® as Treatment for Cancer** 537
Rolf Kalhammer and Natarajan Sethuraman

Mundus Vult Decipi – High Mutation Rates of HIV and New Paradigms for Treatment 549

- 7 **AIDS Gene Therapy: A Vector Selectively Able to Destroy Latently HIV-1-infected Cells** 549
Francisco Luque Vázquez and Ricardo Oya
- 8 **Combinatorial RNA-based Therapies for HIV-1** 569
Kevin V. Morris and John J. Rossi

Part III Improving the Development of Biopharmaceuticals

Citius, Altius, Fortius – Acceleration by High Throughput and Ultra-HT 583

- 1 **Design of Modern Biopharmaceuticals by Ultra-high-throughput Screening and Directed Evolution** 583
Markus Rarbach, Wayne M. Coco, Andre Koltermann, Ulrich Kettling, and Manfred Eigen
- 2 **Learning from Viruses: High-throughput Cloning using the Gateway® System to Transfer Genes without Restriction Enzymes** 605
Jonathan D. Chesnut

In Vivo Veritas – Early Target Validation in Knock-out Mice and More 621

- 3 **Target Validation: An Important Early Step in the Development of Novel Biopharmaceuticals in the Post-genomic Era** 621
Christoph P. Bagowski
- 4 **Genetically Modified Mice in Medical and Pharmaceutical Research** 649
Cord Brakebusch
- 5 **An NIH Model Organism for Biopharmaceutical and Biomedical Research: The Lower Eukaryote *Dictyostelium discoideum*** 661
Thomas Winckler, Ilse Zündorf, and Theodor Dingermann

Revolution by Evolution – Rational Design for Desire and Scientific Art of Optimization 695

- 6 **Releasing the Spring: Cofactor- and Substrate-assisted Activation of Factor IXa** 695
Hans Brandstetter and Katrin Sichler
- 7 **Accelerating Diagnostic Product Development Process with Molecular Rational Design and Directed Evolution** 703
Harald Sobek, Rainer Schmuck, and Zhixin Shao

Volume 3

Part IV Production of Biopharmaceuticals

The Industry's Workhorses – Mammalian Expression Systems 723

- 1 **Manufacture of Recombinant Biopharmaceutical Proteins by Cultivated Mammalian Cells in Bioreactors** 723
Florian M. Wurm
- 1.1 **Introduction** 724

1.2	Vectors, Transfections, and Cell Line Generation	727
1.3	Host Cell Engineering	731
1.4	Gene Transfer and Gene Amplification in Mammalian Cells	733
1.5	Production Principles for Mammalian Cells: Anchorage-dependent Cultures and Suspension Cultures	737
1.6	Large-scale Transient Expression	744
1.7	Regulatory Issues	745
1.8	Concluding Remarks	751
2	Alternative Strategies and New Cell Lines for High-level Production of Biopharmaceuticals	761
	<i>Thomas Rose, Karsten Winkler, Elisabeth Brundke, Ingo Jordan and Volker Sandig</i>	
2.1	Mammalian Cells as a Workhorse to Produce Protein-based Biopharmaceuticals	761
2.2	The Cell Line of Choice	762
2.3	Pushing Expression Levels – Impact of Vector Design and Cell Clone Selection	764
2.4	A Single CHO High-producer Clone for Multiple Products	766
2.5	The G-line: Use of the Immunoglobulin Locus of a Human/Mouse Heterohybridoma for Heterologous Gene Expression	769
2.6	Human Designer Cell Lines	774
2.7	Summary and Conclusion	776
3	PER.C6® Cells for the Manufacture of Biopharmaceutical Proteins	779
	<i>Chris Yallop, John Crowley, Johanne Cote, Kirsten Hegmans-Brouwer, Fija Lagenwerf, Rodney Gagne, Jose Coco Martin, Nico Oosterhuis, Dirk-Jan Opstelten, and Abraham Bout</i>	
3.1	Introduction	780
3.2	Generation of PER.C6 Cells	782
3.3	PER.C6 Cells for the Manufacture of Recombinant Proteins	784
3.4	Fed-batch Process Development	789
3.5	Operation of PER.C6 Cells in Continuous Perfusion	794
3.6	Characterization of Antibodies Produced by PER.C6 Cells	797
3.7	Conclusion	803
4	Use of the Glutamine Synthetase (GS) Expression System for the Rapid Development of Highly Productive Mammalian Cell Processes	809
	<i>John R. Birch, David O. Mainwaring, and Andrew J. Racher</i>	
4.1	Introduction	809
4.2	Cell Line Construction and Selection	810
4.3	Cell Line Stability	818
4.4	Cell Engineering to Increase Productivity	819
4.5	Selection of Useful Cell Sub-populations	822
4.6	Process Development	823
4.7	Summary	830

Vivat, Crescat, Floreat – A Ripe and Blooming Market for Transgenic Animals and Plants 833

5 Biopharmaceuticals Derived from Transgenic Plants and Animals 833

Julio Baez

- 5.1 Introduction 834
- 5.2 Advantages and Disadvantages of Transgenic Systems for the Production of Biopharmaceuticals 845
- 5.3 Commercial Biopharmaceuticals with Human Clinical Experience for Therapeutic, Immunoprophylactic, and Medical Device Use derived from Transgenic Systems 852
- 5.4 Conclusions 873

6 Production of Recombinant Proteins in Plants 893

Victor Klimyuk, Sylvestre Marillonnet, Jörg Knäblein, Michael McCaman, and Yuri Gleba

- 6.1 Introduction 893
- 6.2 Plant-based Expression Systems 894
- 6.3 Plant-made Recombinant Proteins available Commercially, and under Development 903
- 6.4 Comparative Analysis of the Expression Systems and Production Platforms 907
- 6.5 Summary and Conclusion 909

7 Humanized Glycosylation: Production of Biopharmaceuticals in a Moss Bioreactor 919

Gilbert Gorr and Sabrina Wagner

- 7.1 Introduction 919
- 7.2 Mosses: Some General Aspects 920
- 7.3 Cell Culture 922
- 7.4 Recombinant Expression 923
- 7.5 N-Glycosylation 924
- 7.6 Conclusions and Outlook 927

8 ExpressTec: High-level Expression of Biopharmaceuticals in Cereal Grains 931

Ning Huang and Daichang Yang

- 8.1 Introduction 931
- 8.2 Development of ExpressTec for High-level Expression of Recombinant Proteins in Cereal Grains 932
- 8.3 High-level Expression of Biopharmaceuticals in Cereal Grain using ExpressTec 938
- 8.4 Impact of Expression Level on the Cost of Goods 945
- 8.5 Perspectives of Expressing Biopharmaceuticals in High Plants 946

9 Biopharmaceutical Production in Cultured Plant Cells 949

Stefan Schillberg, Richard M. Twyman, and Rainer Fischer

- 9.1 Introduction 950

- 9.2 Recombinant Proteins Produced in Plant Cell Suspension Cultures 951
- 9.3 Challenges and Solutions for the Production of Recombinant Proteins 954
- 9.4 Process Engineering 958
- 9.5 Downstream Processing 959
- 9.6 Regulatory Considerations 960
- 9.7 Conclusions 961

10 Producing Biopharmaceuticals in the Desert: Building an Abiotic Stress Tolerance in Plants for Salt, Heat, and Drought 967

Shimon Gepstein, Anil Grover, and Eduardo Blumwald

- 10.1 General Comments on Abiotic Stresses 968
- 10.2 Drought and Salt Tolerance 969
- 10.3 High-temperature Stress 981
- 10.4 Conclusions and Perspectives 989

11 The First Biopharmaceutical from Transgenic Animals: ATryn® 995

Yann Echelard, Harry M. Meade, and Carol A. Ziomek

- 11.1 Introduction 996
- 11.2 Recombinant Production of AT 998
- 11.3 Characterization of rhAT 1003
- 11.4 Preclinical Studies 1007
- 11.5 Clinical Trials with rhAT 1011
- 11.6 Conclusions 1016

Alea Non lacta Est – Improving Established Expression Systems 1021

12 Producing Modern Biopharmaceuticals: The Bayer HealthCare Pharma Experience with a Range of Expression Systems 1021

Heiner Apeler

- 12.1 The *Escherichia coli* Expression Platform 1022
- 12.2 The *Saccharomyces cerevisiae* Expression Platform 1027
- 12.3 The HKB11 Expression Platform 1029
- 12.4 Outlook and Conclusion 1031

13 Advanced Expression of Biopharmaceuticals in Yeast at Industrial Scale: The Insulin Success Story 1033

Asser Sloth Andersen and Ivan Diers

- 13.1 Introduction 1033
- 13.2 Design and Optimization of the Insulin Precursor Molecule 1036
- 13.3 Production of Insulin 1041
- 13.4 Conclusions and Future Aspects 1042

14 Baculovirus-based Production of Biopharmaceuticals using Insect Cell Culture Processes 1045

Wilfried Weber and Martin Fussenegger

- 14.1 Introduction 1045
- 14.2 Molecular Tools for the Construction of Transgenic Baculoviruses 1046
- 14.3 Insect Cell Culture 1047
- 14.4 Insect Cell Glycosylation and Glycoengineering 1047
- 14.5 Nutrient and Kinetic Considerations for Optimized BEVS-based Protein Production 1048
- 14.6 Scaling-up Baculovirus-based Protein Production 1050
- 14.7 Generic Protocol of Optimized Protein Production 1050
- 14.8 Case study: Rapid Optimization of Expression Conditions and Large-scale Production of a Brutons Tyrosine Kinase Variant (BTK) 1053
- 14.9 Conclusion 1058

15 Robust and Cost-effective Cell-free Expression of Biopharmaceuticals: *Escherichia Coli* and Wheat Embryo 1063

Luke Anthony Miles

- 15.1 Introduction 1064
- 15.2 Transcription 1066
- 15.3 Translational 1068
- 15.4 Treatment of Extracts for Synthesis of Disulfide-bonded Proteins 1072
- 15.5 ATP Regeneration Systems 1074
- 15.6 Reaction Conditions 1075
- 15.7 Conclusion 1079

When Success Raises its Ugly Head – Outsourcing to Uncork the Capacity Bottleneck 1083

16 Contract Manufacturing of Biopharmaceuticals Including Antibodies or Antibody Fragments 1083

J. Carsten Hempel and Philipp N. Hess

- 16.1 Introduction 1084
- 16.2 Expression Systems and Manufacturing Procedures 1085
- 16.3 Outsourcing and Contract Manufacturing 1089
- 16.4 Summary and Outlook 1100

Part V Biopharmaceuticals used for Diagnostics and Imaging

From Hunter to Craftsman – Engineering Antibodies with Nature's Universal Toolbox 1105

1 Thirty Years of Monoclonal Antibodies:

A Long Way to Pharmaceutical and Commercial Success 1105

Uwe Gottschalk and Kirsten Mundt

- 1.1 Introduction 1107
- 1.2 Making Monoclonal Antibodies 1109
- 1.3 Other Antibody Formats: Antibody Fragments 1113

1.4	Medical Application Areas for MABs	1116
1.5	From Initial Failure to Success: Getting the Target Right	1117
1.6	The Market Perspective	1119
1.7	Drug Targeting: The Next Generation in Cancer Treatment	1122
1.8	Developing a Manufacturing Process for MABs	1126
1.9	Routine Manufacture of MABs	1127
1.10	Glycosylation and Other Post-translational Modifications	1132
1.11	Emerging Issues in MAB Production	1134
1.12	The Future of MABs	1136
2	Modern Antibody Technology: The Impact on Drug Development	1147
	<i>Simon Moroney and Andreas Plückthun</i>	
2.1	Introduction	1147
2.2	Immunogenicity	1148
2.3	Technology	1153
2.4	Reaching the Target: The Importance of Specificity, Affinity and Format	1163
2.5	Exerting an Effect at the Target	1168
2.6	Antibodies in their Natural Habitat: Infectious Diseases	1175
2.7	Opportunities for New Therapeutic Applications Provided by Synthetic Antibodies	1176
2.8	Future Directions and Concluding Statements	1177
3	Molecular Characterization of Autoantibody Responses in Autoimmune Diseases: Implications for Diagnosis and Understanding of Autoimmunity	1187
	<i>Constanze Breithaupt</i>	
3.1	Autoantibodies in Autoimmune Diseases	1188
3.2	Autoantibody Epitopes	1190
3.3	Visualization of Epitopes	1195
3.4	Structural Characterization of Autoantibody–Autoantigen Complexes	1199
3.5	Conclusions	1205
	Find, Fight, and Follow – Target-specific Troika from Mother Nature's Pharmacopoeia	1211
4	Molecular Imaging and Applications for Pharmaceutical R&D	1211
	<i>Joke G. Orsel and Tobias Schaeffter</i>	
4.1	Introduction	1212
4.2	Imaging Modalities and Contrast Agents	1213
4.3	Molecular Imaging	1225
4.4	Molecular Imaging for Drug Discovery and Development	1230
4.5	Concluding Remarks	1239

5	Design and Development of Probes for <i>In vivo</i> Molecular and Functional Imaging of Cancer and Cancer Therapies by Positron Emission Tomography (PET)	1243
	<i>Eric O. Aboagye</i>	
5.1	What is Positron Emission Tomography?	1244
5.2	Radiochemistry Considerations	1246
5.3	Pharmacological Objectives in Oncology Imaging Studies	1249
5.4	The Use of Radiolabeled Drugs to Image Tumor and Normal Tissue Pharmacokinetics	1250
5.5	Pharmacodynamic Studies	1254
5.6	Conclusions	1264
6	Ligand-based Targeting of Disease: From Antibodies to Small Organic (Synthetic) Ligands	1271
	<i>Michela Silacci and Dario Neri</i>	
6.1	Introduction	1272
6.2	Ligands	1273
6.3	Classes of Diseases	1276
6.4	From a Ligand to a Product	1288
6.5	Concluding Remarks	1289
7	Ultrasound Theranostics: Antibody-based Microbubble Conjugates as Targeted <i>In vivo</i> Contrast Agents and Advanced Drug Delivery Systems	1301
	<i>Andreas Briel, Michael Reinhardt, Mathias Mäurer, and Peter Hauff</i>	
7.1	Motivation: "Find, Fight and Follow!"	1302
7.2	Ultrasound: "Hear the Symptoms"	1304
7.3	Ultrasound Contrast: "Tiny Bubbles"	1305
7.4	The Perfect Modality: "Sensitive Particle Acoustic Quantification (SPAQ)"	1308
7.5	Targeting and Molecular Imaging: "The Sound of an Antibody"	1309
7.6	Drug Delivery: "The Magic Bullet"	1315
7.7	Ultrasound, Microbubbles and Gene Delivery: "Noninvasive Micro-Gene Guns"	1318
7.8	Summary: Ultrasound Theranostics "Building a Bridge between Therapy and Diagnosis"	1320
	Getting Insight – Sense the Urgency for Early Diagnostics	1325
8	Development of Multi-marker-based Diagnostic Assays with the ProteinChip® System	1325
	<i>Andreas Wiesner</i>	
8.1	The Urgency of Earlier Diagnosis	1326
8.2	Proteins are Best Choice Again	1327
8.3	Current Tools for Protein Biomarker Detection	1328
8.4	The ProteinChip® System at a Glance	1329
8.5	Distinctions of the SELDI Process	1333

- 8.6 The Pattern Track™ Process:
From Biomarker Discovery to Assay Development 1334
- 8.7 Protein Variants as Disease Markers 1337
- 8.8 Conclusion and Outlook 1338

- 9 Early Detection of Lung Cancer: Metabolic Profiling of Human Breath with Ion Mobility Spectrometers 1343
Jörg Ingo Baumbach, Wolfgang Vautz, Vera Ruzsanyi, and Lutz Freitag
- 9.1 Introduction 1343
- 9.2 Material and Methods: IMS 1345
- 9.3 Results and Discussion 1347
- 9.4 Clinical Study 1349
- 9.5 Conclusions 1354

Volume 4

Part VI Advanced Application Routes for Biopharmaceuticals

Getting Inside – Quest for the Best and How to Improve Delivery 1361

- 1 Advanced Drug Delivery Systems for Biopharmaceuticals 1361
Gesine E. Hildebrand and Stephan Harnisch

Pathfinder – New Ways for Peptides, Proteins and Co 1393

- 2 Poly(ethylene) Glycol Conjugates of Biopharmaceuticals in Drug Delivery 1393
Michael D. Bentley, Mary J. Bossard, Kevin W. Burton, and Tacey X. Viegas
- 3 Novel Vaccine Adjuvants Based on Cationic Peptide Delivery Systems 1419
Karen Lingnau, Christoph Klade, Michael Buschle, and Alexander von Gabain
- 4 The Evolving Role of Oralin™ (Oral Spray Insulin) in the Treatment of Diabetes using a Novel RapidMist™ Diabetes Management System 1445
Pankaj Modi
- 5 Improvement of Intestinal Absorption of Peptide and Protein Biopharmaceuticals by Various Approaches 1463
Akira Yamamoto

Via Mala – the Stoney Road of DNA Delivery: Back-pack, Feed-back, and Pay-back 1487

- 6 DNA Vaccine Delivery from Poly(ortho ester) Microspheres 1487
Chun Wang, Herman N. Eisen, Robert Langer, and Jorge Heller

- 7 **Liposomal *In vivo* Gene Delivery** 1507
Shigeru Kawakami, Fumiyoshi Yamashita, and Mitsuru Hashida
- 8 **Programmed Packaging:
A New Drug Delivery System and its Application to Gene Therapy** 1521
Kentaro Kogure, Hidetaka Akita, Hiroyuki Kamiya, and Hideyoshi Harashima

Getting Beyond – Rocket Science vs. Science Fiction 1537

- 9 **Bionanotechnology and its Role to Improve Biopharmaceuticals** 1537
Oliver Kayser

Part VII From Transcription to Prescription of Biopharmaceuticals

Dosis Facit Venenum – The Therapeutic Window between Systemic Toxicity and Lack of Efficacy 1557

- 1 **Analytics in Quality Control and *In vivo*** 1557
Michael Hildebrand
- 2 **Design, Development and Optimization: Crystal Structures
of Microsomal Cytochromes P450** 1581
*Dijana Matak Vinković, Sheena Whyte, Harren Jhoti, Jose Cosme,
and Pamela A. Williams*
- 3 **Mettox™: A Suite of Predictive *In silico* and *In vitro* Assays for Metabolic
and Genotoxicological Profiling of Preclinical Drug Candidates** 1603
Michael Murray

Happy End: Claim to Fame and Approval 1637

- 4 **Considerations for Developing Biopharmaceuticals: FDA Perspective** 1637
*Kurt Brorson, Patrick G. Swann, Janice Brown, Barbara Wilcox,
and Marjorie A. Shapiro*
- 5 **The Regulatory Environment for Biopharmaceuticals in the EU** 1669
Axel F. Wenzel and Carina E. A. Sonnega

Part VIII From Bench to Bedside – The Aftermaths

Think Big and Dealmaking for Growth – Global Changes in the Health-care Sector 1711

- 1 **Healthcare Trends and their Impact on the Biopharmaceutical Industry:
Biopharmaceuticals Come of Age** 1711
Alexander Moscho, Markus A. Schäfer, and Kristin Yarema