

Contents

Introduction and Preface	XIII
List of Contributors	XVII
Abbreviations	XXI
List of Boxes and Definitions	XXXI

1	Yeast-Based Chemical Genomic Approaches	1
	<i>Katja Hübel</i>	
1.1	Introduction	1
1.2	The Biological Problem	1
1.2.1	Interplay between Organic Chemistry and Biology	1
1.2.2	Chemical Genomics	4
1.2.3	Small Molecules in Chemical Genomics	6
1.2.4	Cell-based Genomic Approaches	6
1.2.5	Yeast-Based Chemical Genomic Approaches	7
1.3	The Chemical Approach	11
1.4	Chemical Biological Research/Evaluation–Chemogenomic Profiling: Elucidating the Mode of Action of Small Molecules	12
1.4.1	Assay Principle	12
1.4.2	The Yeast Deletion Strain Collection	14
1.4.2.1	Homozygous Deletion Strains	14
1.4.2.2	Heterozygous Deletion Strains	14
1.4.3	Advantages and Disadvantages	16
1.4.4	Case Studies	16
1.5	Conclusions	19
	References	19
2	Microarray-Based Strategies to Identify Unknown Protein Interactions	21
	<i>Sabine Borgmann and Christof M. Niemeyer</i>	
2.1	Introduction	21
2.2	The Biological Problem	21
2.3	The Chemical Approach	25
2.3.1	Introduction to Microarray Technology	25

2.3.2	Antibody Microarray for Protein Profiling	28
2.3.3	Self-Assembled Protein Microarrays	29
2.3.4	Self-Assembled Small-Molecule Microarrays	30
2.4	Chemical Biological Research/Evaluation	33
2.4.1	Antibody Microarray for Protein Profiling	33
2.4.2	Self-Assembled Protein Microarrays	35
2.4.3	Self-Assembled Small-Molecule Microarrays	36
2.5	Conclusions	37
	References	37
3	Compound Collection Synthesis for Chemical Biology	39
	<i>Kamal Kumar</i>	
3.1	Introduction	39
3.2	Chemical Probes—a Chemical Question with Biological Consequences	39
3.3	Diversity Oriented Synthesis (DOS)	40
3.3.1	Aims of Diversity Oriented Synthesis	40
3.3.2	DOS Based Library Synthesis and Evaluation	42
3.4	Biology Oriented Synthesis (BIOS)	46
3.4.1	Aims of Biology Oriented Synthesis	46
3.4.2	BIOS-Based Library Synthesis and Evaluation	47
3.5	Conclusions	52
	References	52
4	Target Identification and Validation of a WNT/β-Catenin Pathway Modulator	55
	<i>Petra Janning</i>	
4.1	Introduction	55
4.2	The Biological Problem	55
4.2.1	Target Identification and Validation	55
4.2.2	WNT/ β -Catenin Pathway	56
4.2.3	Modulation of the WNT/ β -Catenin Pathway by Small Molecules	57
4.3	The Chemical Approach	59
4.3.1	High Throughput Screens	59
4.3.2	Synthesis of QS11, QS11-NC and Coupling to Solid Support	60
4.4	Chemical Biological Research/Evaluation	62
4.4.1	Target Identification	62
4.4.2	Target Validation	64
4.4.3	Mechanism of Action	65
4.5	Conclusions	66
	References	66
5	Activity-Based Protein Profiling of Cys Proteases	69
	<i>Renier van der Hoorn</i>	
5.1	Introduction	69
5.2	The Biological Problem	69

5.3	The Chemical Approach	70
5.3.1	Epoxide Probes for Papain-Like Cys Proteases	71
5.3.2	Acyloxymethylketones for Caspase-like Proteases	72
5.3.3	Vinyl Sulfones for Ubiquitin-Specific Proteases	74
5.4	Chemical Biological Research/Evaluation	75
5.5	Conclusions	78
	References	78
6	The Use of Photoaffinity Labeling for the Identification of the Binding Site of the Antibiotic Linezolid	79
	<i>Rolf Breinbauer and Matthias Mentel</i>	
6.1	Introduction	79
6.2	The Biological Problem	79
6.3	The Chemical Approach	81
6.3.1	Synthesis of Oxazolidinone Antibiotics	82
6.3.2	Synthesis of Photoaffinity-Labeled Probes	83
6.4	Chemical Biological Research/Evaluation	86
6.5	Conclusions	87
	References	88
7	Surgical Strikes: Uses of Analog Sensitive Technologies to Target Kinases of Interest	89
	<i>Matthias Rabiller and Daniel Rauh</i>	
7.1	Introduction	89
7.2	The Biological Problem	89
7.3	The Chemical Genetic Approach	91
7.3.1	Aim of the Chemical Genetic Approach	91
7.3.2	Engineering ASKA Ligand–Kinase Pairs	95
7.4	Chemical Biological Research/Evaluation: ASKA Technology–Applications in Molecular Biology	99
7.4.1	Kinase Substrate Identification	99
7.4.2	Kinase Inhibition Studies	101
7.5	Conclusions	102
	References	102
8	Modulation of Protein–Protein Interactions by Small Molecules	105
	<i>Stefanie Bovens and Christian Ottmann</i>	
8.1	Introduction	105
8.2	The Biological Problem	105
8.3	The Chemical Approach	111
8.4	Chemical Biological Research/Evaluation	114
8.4.1	Homodimerization	114
8.4.2	Heterodimerization	116
8.4.3	Engineering the Protein–Ligand Interaction	117
8.5	Conclusions	118
	References	119

9	Targeted Protein Degradation in Live Cells	121
	<i>Markus Kaiser</i>	
9.1	Introduction	121
9.2	The Biological Problem	121
9.3	The Chemical Approach	124
9.3.1	The PROTAC Approach	124
9.3.2	Conditional Control of Engineered Protein Stability	127
9.4	Chemical Biological Research/Evaluation	129
9.4.1	<i>In Vivo</i> Targeted Protein Degradation by PROTACs	129
9.4.2	<i>In Vivo</i> Evaluation of Shld-1-Controlled Degradation of DD-POI Fusion Proteins	130
9.5	Conclusions	131
	References	132
10	Trapoxin B: From Total Synthesis to Epigenetics	135
	<i>Bruno Bulic</i>	
10.1	Introduction	135
10.2	The Biological Problem	135
10.3	The Chemical Approach	138
10.3.1	Properties of Trapoxin B	138
10.3.2	Synthesis of the K-trap Affinity Matrix	139
10.4	Chemical Biological Research/Evaluation	140
10.5	Conclusions	143
	References	144
11	Native Chemical Ligation—A Tool for Chemical Protein Synthesis	145
	<i>Christian F.W. Becker</i>	
11.1	Introduction	145
11.2	The Synthetic Challenge	145
11.3	The Chemical Approach—Native Chemical Ligation	148
11.3.1	Principles of Native Chemical Ligation	148
11.3.2	Challenges and Limitations	149
11.4	Chemical Biological Research/Evaluation—The Ras-RBD System	150
11.4.1	Chemical Synthesis of the Ras Protein and Its Effector RBD	152
11.4.2	<i>In Vitro</i> Analysis of Synthetic Ras and RBD	153
11.5	Conclusions	156
	References	156
12	Using Split Inteins to Prepare Semi-synthetic Proteins and to Study the Mechanism of Protein <i>trans</i>-Splicing	159
	<i>Henning D. Mootz</i>	
12.1	Introduction	159
12.2	The Biological Problem	159
12.3	The Chemical Approach	160
12.3.1	Traditional Protein Bioconjugation	160
12.3.2	Reprogramming Protein Biosynthesis	161

12.3.3	Chemical Ligation Methods for Protein Semi-synthesis	161
12.3.4	Chemoenzymatic Ways to Ligate Two Polypeptides	163
12.4	Chemical Biological Research/Evaluation	164
12.4.1	Mechanism and Structure of Inteins and Split Inteins	164
12.4.2	Semi-synthesis Mediated by Protein <i>trans</i> -Splicing	166
12.4.3	Investigation of the Mechanism of Protein Splicing Using a Semi-synthetic Intein	171
12.5	Conclusions	172
	Acknowledgements	173
	References	173
13	Elucidation of the Ras Cycle with Semi-synthetic Proteins	175
	<i>Luc Brunsveld</i>	
13.1	Introduction	175
13.2	The Biological Problem	175
13.3	The Chemical Approach	178
13.3.1	Synthesis of Lipidated Ras-peptides	178
13.3.2	Synthesis of Ras-proteins	181
13.4	Chemical Biological Research/Evaluation	183
13.5	Conclusions	186
	References	186
14	The Study of Rab GTPase Recycling Using Semi-synthetic Prenylated Proteins	189
	<i>Kirill Alexandrov, Roger Goody, Herbert Waldmann, and Yaowen Wu</i>	
14.1	Introduction	189
14.2	The Biological Problem	189
14.3	The Chemical Approach	192
14.3.1	Expressed Protein Ligation	192
14.3.2	Synthesis of Prenylated Peptides	192
14.3.3	Semi-synthesis of Soluble Prenylated Rab Proteins	194
14.4	Biological Research/Evaluation	197
14.4.1	Structure of Mono- and Di-geranylgeranylated Ypt1:GDI Complexes	197
14.4.2	Quantitative Analysis of the Interaction between Prenylated Rab and REP/GDI	200
14.4.3	Mechanistic Model of REP/GDI-Mediated Membrane Extraction of Rab Proteins	201
14.5	Conclusions	204
	References	205
15	Development of a Model Cancer Vaccine Candidate Based on the MUC1 Cellular Surface Glycopeptide Epitope	207
	<i>Christian Hedberg and Ulrika Westerlind</i>	
15.1	Introduction	207
15.2	The Biological Problem	207

15.2.1	Carbohydrates in General	207
15.2.2	Mucin 1 and Aberrant Glycosylation in Cancer	208
15.2.3	Immunology	210
15.3	The Chemical Approach	210
15.3.1	Synthesis of Glycosylated Amino Acid Building Blocks	212
15.3.2	Synthesis of MUC1 Glycopeptides Conjugated to a Peptide OVA _{323–339} T-Cell Epitope	214
15.3.3	Synthesis of MUC1 Glycopeptides Conjugated to BSA Carrier Protein for ELISA	214
15.4	Chemical Biological Research/Evaluation	217
15.5	Conclusions	217
	References	219
16	The Introduction of Chemical Reporter Groups by Bioorthogonal Ligation Reactions for the Imaging of Cell-Surface Glycans	221
	<i>Rolf Breinbauer and Matthias Mentel</i>	
16.1	Introduction	221
16.2	The Biological Problem	221
16.3	The Chemical Approach	222
16.3.1	Preparation of Cell-Surface Glycans Exhibiting an Azide Moiet	222
16.3.2	Synthesis of Alkyne-Tagged Fluorescent Probes	225
16.4	Chemical Biological Research/Evaluation	228
16.5	Conclusions	229
	References	232
17	Sequence Specific DNA-Binding Small Molecules for Protein Recruitment and Modulation of Transcription	233
	<i>Hans-Dieter Arndt and Sascha Baumann</i>	
17.1	Introduction	233
17.2	The Biological Problem—Targeting DNA with Small Molecules	233
17.3	The Chemical Approach: The Polyamide Rationale	234
17.4	Chemical Biological Research/Evaluation—Polyamides in Biological Applications	237
17.4.1	Hypoxia Inducible Factor	237
17.4.2	Artificial Activators	240
17.5	Conclusions	243
	References	243
18	G-Quadruplex Ligands as Tools for Elucidating c-MYC Transcriptional Regulation	247
	<i>Barbara Saccà and Markus Kaiser</i>	
18.1	Introduction	247
18.2	The Biological Problem	247
18.3	The Chemical Approach	251

18.4	Chemical Biological Research/Evaluation	254
18.5	Conclusion	258
	References	258
	Index	261