Contents

Preface xiii
A Personal Foreword xvii

1 Chemical Strategies for Evaluating New Drug Targets 1
Adrian J. Carter, Raina Seupel, Paul E. Brennan, Michael Sundström, Andrea Introini, and Anke Mueller-Fahrnow
1.1 Introduction 1
1.2 Use Cases and Case Studies for Chemogenomic Compounds and Chemical Probes 5
1.2.1 Chemogenomic Libraries 5
1.2.2 Inactive Control 6
1.2.3 Use of Biological Target Panels and Profiling 8
1.3 Development of Chemical Probes 10
1.3.1 From BIX01294 to EPZ035544: Development and Improvement of G9a/GLP Inhibitors 10
1.3.2 Development of BRD9 Inhibitors 12
1.4 Compound-Based Target Evaluation with Patient-Derived Cells 14
1.4.1 Compound-Based Target Evaluation 14
1.4.2 Patient-Derived Cell Assays 16
1.4.3 Target Evaluation Approach 16
1.4.4 Case Story: Inflammatory Bowel Disease (IBD) Tissue Platform 18
1.5 Summary and Outlook 19
References 20

2 Affinity-Based Chemoproteomics for Target Identification 25
Annika Jenmalm Jensen and Ivan Cornella Taracido
2.1 Introduction 25
2.2 Small Molecule Phenotypic Mechanism of Action Elucidation 29
2.3 Quantitative High-Resolution Mass Spectrometry as a Protein Detection Read-Out 30
2.4 In-Lysate Affinity-Based Chemical Proteomics 33
2.4.1 Design of the Affinity Probe 34
2.4.2 General Experimental Pulldown Workflow 36
2.4.3 Limitations 38
3 Activity-Based Protein Profiling  51
Nattawadee Panyain, Cassandra R. Kennedy, Ryan T. Howard, and Edward W. Tate

3.1 Introduction  51
3.2 Activity-Based Probe (ABP) and Affinity-Based Probe (AfBP) Design  53
3.2.1 Warheads (Reactive Groups)  53
3.2.1.1 Electrophilic Warheads  55
3.2.1.2 Photocrosslinking Warheads  55
3.2.2 Reporter Tags  56
3.2.3 Linkers  56
3.2.4 Bioorthogonal Ligation Chemistry  57
3.2.4.1 Staudinger Ligation  58
3.2.4.2 Copper(I)-Catalysed Azide-Alkyne Cycloaddition (CuAAC)  58
3.2.4.3 Strain-Promoted Azide-Alkyne Cycloaddition (SPAAC)  59
3.2.4.4 Diels–Alder Reaction  59
3.3 Chemical Proteomic Workflow  60
3.3.1 Quantitative Proteomics by Mass Spectrometry  61
3.3.1.1 Label-Free Quantification (LFQ)  61
3.3.1.2 Chemical Labelling Quantification  61
3.3.1.3 Metabolic Labelling Quantification  63
3.4 ABPP Applications and Case Studies  63
3.4.1 Case Study 1: Activity-Based Protein Profiling as a Robust Method for Enzyme Identification and Screening in Extremophilic Archaea  65
3.4.2 Case Study 2: Failed Clinical Trial of a Fatty Acid Amide Hydrolase (FAAH) Inhibitor  68
3.4.3 Case Study 3: Target Identification of Small Molecule Inhibitors  71
3.4.3.1 New Target Profiling for Sulforaphane  71
3.4.3.2 Profiling USP Inhibitors in Human Cell Lines as Potential Therapeutic Molecules  73
3.4.4 Case Study 4: Fragment-Based Ligand Discovery Aided by Photoaffinity Labelling  74
3.4.5 Case Study 5: Quenched Fluorescent Activity-Based Probe (qABP) Design and Application in Protein Localization  80
3.5 Summary  82
References  83
4 Kinobeads: A Chemical Proteomic Approach for Kinase Inhibitor Selectivity Profiling and Target Discovery 97
Maria Reinecke, Stephanie Heinzlmeir, Mathias Wilhelm, Guillaume Médard, Susan Klaeger, and Bernhard Kuster

4.1 Kinase Inhibitor Target Deconvolution Using Chemical Proteomics 97
4.1.1 Polypharmacology of Small Molecule Kinase Inhibitors 97
4.1.2 Chemoproteomic Profiling of Kinase Inhibitors 100
4.1.3 Tips and Tricks Regarding Chemoproteomic Assay Development 103

4.2 Detailed Kinobeads Protocol 105
4.2.1 Cell or Tissue Lysate 107
4.2.2 Affinity Matrices 107
4.2.3 Kinobeads Competition Assay 110
4.2.4 Mass Spectrometry 111
4.2.5 Peptide and Protein Identification and Quantification 112
4.2.6 Data Analysis 112

4.3 Application Examples for Kinobeads 113
4.3.1 Expanding the Target Space of Kinobeads 113
4.3.2 Target Space Deconvolution of Small Molecule Kinase Inhibitors 116
4.3.3 Opportunities Arising from Inhibitor Polypharmacology: Drug Repositioning 120
4.3.4 Chemoproteomic-Guided Medicinal Chemistry 121

4.4 Kinobeads, Inhibitors, and Drug Discovery: Where Are We Heading? 123
4.4.1 What Is a Good Drug? 123
4.4.2 How Can We Discover New Drugs in the Future? 124
4.4.3 The Yin and Yang of Chemoproteomic-Guided Drug Discovery 124

Acknowledgments 125
References 125

5 Label-Free Techniques for Target Discovery and Validation 131
Daniel Martinez Molina and Michael Dabrowski

5.1 Introduction 131
5.2 CETSA: How It All Began 132
5.3 The CETSA Formats 136
5.3.1 CETSA Classics 136
5.3.2 CETSA HT 138
5.3.3 CETSA MS 140

5.4 Target Discovery 142
5.4.1 Generation of Active Hit Molecules 142
5.4.2 Tool Generation (Small Screens to Identify Tool Compounds) 143
5.4.3 Target Classes That Are In and Out of Scope and Difficult Targets 143
5.4.4 Focused or Iterative Library Screening 144
5.4.5 Fragment Library Screening 144
5.4.6 Hit Confirmation 145
5.4.7 Phenotypic Hit Deconvolution to Discover Targets 145
5.5 Target Validation 147
5.5.1 Binding Modes 147
5.5.2 Selectivity, Specificity, and Safety 148
5.5.3 Translation Bench to Bedside (via Animals) 149
5.6 Conclusion 150

References 151

6 Reverse Translation to Support Efficient Drug Target Selection and Stratified Medicine 153
Lauren Drowley and Martin Armstrong
6.1 Introduction: the Challenge 153
6.2 Genetics to Date in Drug Discovery 154
6.3 Genetic Strategies for Target Discovery 156
6.3.1 GWAS 158
6.3.2 Rare Disease Genetics 160
6.3.2.1 Rare Mutation → Rare Disease Drug Discovery 161
6.3.2.2 Rare Mutation → Common Disease Drug Discovery 161
6.3.3 Somatic Mutations 162
6.3.4 Analytical Approaches 163
6.4 Functional Validation 164
6.4.1 Prioritization of Putative Mutations 165
6.4.2 Determining Functional Consequence of Mutation 165
6.4.2.1 Publicly Available Data 165
6.4.2.2 Systems Biology 166
6.4.2.3 Model Systems: ‘The Tissue Is the Issue’ 168
6.4.3 Druggability: From Validation of a Gene to a Druggable Target 169
6.5 Forward-Looking Perspectives 170
6.5.1 Molecular Taxonomy of Disease 171
6.5.2 Precision Medicine 171
6.5.3 Data Integration 172
6.6 Conclusion 173

References 173

7 Elucidating Target Biology and Drug Mechanism of Action Across Human Cell-Based Model Systems 179
John C. Dawson and Neil O. Carragher
7.1 Introduction 179
7.2 Advances in Human Cell-Based Model Development 182
7.2.1 Next-Generation Sequencing (NGS) 183
7.2.2 CRISPR Genome Editing 184
7.2.3 Induced Pluripotent Stem Cell Biology 184
7.2.4 3D Cell and Organoid Models 185
7.2.5 Microfluidic and Organ-on-a-Chip Devices 186
7.2.6 In Vivo Imaging 188
7.2.7 High-Content Imaging  190
7.3 Multiparametric High-Content Phenotypic Profiling of Target Biology and Drug Mechanism of Action  191
7.3.1 High-Content Cell Painting in Functional Genomics  193
7.3.2 Integration of Multiparametric High-Content Imaging with Chemoinformatics  195
7.3.3 Guiding Chemical Design and Target Selectivity from Multiparametric High-Content Analysis  195
7.4 Target-Annotated Compound Libraries for Phenotypic Screening and MOA Determination  196
7.5 Quantitative Pathway Profiling Across New Model Systems  197
7.5.1 Pathway Profiling at the Gene Transcription Level  198
7.5.2 Dynamic Post-Translational Pathway Profiling Across Dose–Response and Time-Series Studies  199
7.6 Conclusions  202
References  203

8 Cell Biology Methods in Target Validation  211
Manfred Köegl and Simon Wöhre  
8.1 Introduction  211
8.2 Biomarkers  211
8.2.1 Direct Target Engagement Biomarkers  212
8.2.2 Indirect Target Engagement Biomarkers and Pathway Biomarkers  213
8.2.3 Response Biomarkers  214
8.2.4 Correlation of Biomarkers  214
8.3 Direct Evidence to Show That Modulation of a Target Leads to a Cellular Response  219
8.4 Direct Evidence That Target Modulation Is Responsible for Cellular Responses by Mutations Conferring Sensitivity to Existing Drugs  219
8.4.1 The ‘Bump-and-Hole’ Approach to Generate Sensitivity to Small Molecule Inhibitors  219
8.4.2 Chemogenomic Approaches for Inducible Degradation of Protein Targets  222
8.5 Resistance Conferring Mutations  226
References  229

9 Genetic Manipulation/Modulation for Target Discovery and Validation  233
Christophe Lanneau, Georges Kalouche, Xinning Cai, Francois Lo-Presti, and Christoph Potting
9.1 Introduction  233
9.2 Overview of the Development of Leading Genetic Manipulation Technologies  234
9.2.1 RNAi, ZFNs, and TALENs  234
9.2.2 Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)  237
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.3 Considerations for Designing and Interpreting CRISPR Experiments</td>
<td>238</td>
</tr>
<tr>
<td>9.3.1 Methodological Considerations for Genetic Manipulation by the</td>
<td>238</td>
</tr>
<tr>
<td>CRISPR/Cas Technology</td>
<td></td>
</tr>
<tr>
<td>9.3.2 Choosing a Cellular Model: Biological and Genomic Aspects</td>
<td>239</td>
</tr>
<tr>
<td>9.3.3 gRNA Design</td>
<td>242</td>
</tr>
<tr>
<td>9.3.3.1 Identification of Target Locations</td>
<td>242</td>
</tr>
<tr>
<td>9.3.3.2 Selection of Spacer Sequences</td>
<td>245</td>
</tr>
<tr>
<td>9.3.3.3 Predictive Tools</td>
<td>247</td>
</tr>
<tr>
<td>9.3.4 Successful Application of the CRISPR/Cas Technology</td>
<td>249</td>
</tr>
<tr>
<td>9.3.4.1 Delivering CRISPR Reagents to Target Cells</td>
<td>249</td>
</tr>
<tr>
<td>9.3.4.2 Check for Anticipated Knockout/Knock-In</td>
<td>252</td>
</tr>
<tr>
<td>9.4 Further Developments of the CRISPR/Cas Technology Facilitates</td>
<td>253</td>
</tr>
<tr>
<td>Additional Modes of Genetic Perturbation</td>
<td></td>
</tr>
<tr>
<td>9.4.1 CRISPRi</td>
<td>253</td>
</tr>
<tr>
<td>9.4.2 CRISPRa</td>
<td>253</td>
</tr>
<tr>
<td>9.4.3 Base Editing</td>
<td>254</td>
</tr>
<tr>
<td>9.5 The CRISPR/Cas Technology in Target Discovery and Validation</td>
<td>254</td>
</tr>
<tr>
<td>9.5.1 CRISPR/Cas Technology for Early Target Validation</td>
<td>254</td>
</tr>
<tr>
<td>9.5.2 CRISPR Screens and Use for Target Discovery</td>
<td>255</td>
</tr>
<tr>
<td>9.5.3 CRISPR Screens: General Principle and Considerations</td>
<td>256</td>
</tr>
<tr>
<td>9.5.4 Selected Examples of Target Discovery Using CRISPR Screens to</td>
<td>258</td>
</tr>
<tr>
<td>Illustrate the Breadth of Applications</td>
<td></td>
</tr>
<tr>
<td>9.6 Application of CRISPR Genome Editing in Immunology Studies</td>
<td>260</td>
</tr>
<tr>
<td>9.7 Concluding Remarks</td>
<td>262</td>
</tr>
<tr>
<td>References</td>
<td>263</td>
</tr>
<tr>
<td>10 Computational Approaches for Target Inference</td>
<td>277</td>
</tr>
<tr>
<td>Gerhard Hessler, Christoph Grebner, and Hans Matter</td>
<td></td>
</tr>
<tr>
<td>10.1 Introduction</td>
<td>277</td>
</tr>
<tr>
<td>10.2 Data Annotation for Target Identification</td>
<td>278</td>
</tr>
<tr>
<td>10.3 In Silico Methods for Target Identification</td>
<td>280</td>
</tr>
<tr>
<td>10.3.1 2D Similarity Methods for Target Inference</td>
<td>283</td>
</tr>
<tr>
<td>10.3.2 3D Similarity Methods for Target Inference</td>
<td>289</td>
</tr>
<tr>
<td>10.3.3 Fragment-Based Approaches</td>
<td>290</td>
</tr>
<tr>
<td>10.3.4 QSAR Models and Machine Learning</td>
<td>292</td>
</tr>
<tr>
<td>10.3.5 Experimentally Derived Molecular Descriptors</td>
<td>297</td>
</tr>
<tr>
<td>10.3.6 Structure-Based Screening</td>
<td>299</td>
</tr>
<tr>
<td>10.3.7 Protein–Protein and Ligand–Target Networks</td>
<td>302</td>
</tr>
<tr>
<td>10.4 Practical Considerations</td>
<td>304</td>
</tr>
<tr>
<td>10.5 Conclusion</td>
<td>307</td>
</tr>
<tr>
<td>References</td>
<td>308</td>
</tr>
<tr>
<td>11 Bioinformatic Approaches in the Understanding of Mechanism of Action</td>
<td>323</td>
</tr>
<tr>
<td>(MoA) Maria-Anna Trapotsi, Ian Barrett, Ola Engkvist, and Andreas</td>
<td></td>
</tr>
<tr>
<td>Bender</td>
<td></td>
</tr>
<tr>
<td>11.1 Bioinformatics: Introduction</td>
<td>323</td>
</tr>
</tbody>
</table>
11.1.1 Some Definitions: Mechanism Versus Mode of Action 323
11.1.2 Importance of MoA and Target Prediction in the Drug Discovery Process 324
11.1.3 Different Levels of Information in Mechanism of Action and Target Prediction 325
11.2 Transcriptomics Data and Databases 326
11.2.1 Biological Background of the Transcription Process 326
11.2.2 Connectivity Map: CMap 327
11.2.2.1 Applications of CMap in MoA Deconvolution 328
11.2.3 Library of Integrated Network-Based Cellular Signatures (LINCS) 331
11.2.3.1 LINCS L1000 Data Exploration 332
11.2.3.2 Applications of L1000 Data in MoA Understanding 333
11.3 Pathway Data and Databases 339
11.3.1 What Is a Pathway? 339
11.3.2 Process of Pathway Analysis 341
11.3.3 Pathways in the Understanding of MoA 345
11.3.3.1 Methodology 1: MoA Analysis by Annotating Predicted Compounds’ Targets with Pathways 345
11.3.4 Combination of Gene Expression and Pathway Data 346
11.3.4.1 Methodology 2: Construction of Drug Networks (DNs) with Gene Expression Data and Pathway Annotations 346
11.3.4.2 Methodology 3: Link Drug Target and Pathway Activation to Understand MoA 347
11.4 Image-Based Data 348
11.4.1 Image Data and Where to Extract Them From 348
11.4.2 Application of Image-Based Data in Target Prediction and Better Understanding of MoA 350
11.4.2.1 Methodology 1: Clustering of Compounds Based on Cell Morphology 350
11.4.2.2 Methodology 2: Use of Image-Based Data in the Development of a Cell Morphology Database That Can Facilitate Drug Target Identification 350
11.4.2.3 Methodology 3: Use of Image Data in Drug Repositioning and Biological Activity Prediction 353
11.4.2.4 Methodology 4: Association of Genes with Context-Dependent Morphology Alterations from Cells Exposed to Chemical or Genetic Perturbations for MoA Elucidation 354
11.5 Conclusions 357
Acknowledgement 357
References 357

Index 365