Contents

Preface XV

A Personal Foreword XVII

List of Contributors XIX

Part I: Concept and Theory

1 The Concept of Fragment-based Drug Discovery 3
Daniel A. Erlanson and Wolfgang Jahnke
1.1 Introduction 3
1.2 Starting Small: Key Features of Fragment-based Ligand Design 4
1.2.1 FBS Samples Higher Chemical Diversity 4
1.2.2 FBS Leads to Higher Hit Rates 5
1.2.3 FBS Leads to Higher Ligand Efficiency 6
1.3 Historical Development 6
1.4 Scope and Overview of this Book 7
References 9

2 Multivalency in Ligand Design 11
Vijay M. Krishnamurthy, Lara A. Estroff, and George M. Whitesides
2.1 Introduction and Overview 11
2.2 Definitions of Terms 12
2.3 Selection of Key Experimental Studies 16
2.3.1 Trivalency in a Structurally Simple System 17
2.3.2 Cooperativity (and the Role of Enthalpy) in the “Chelate Effect” 18
2.3.3 Oligovalency in the Design of Inhibitors to Toxins 18
2.3.4 Bivalency at Well Defined Surfaces (Self-assembled Monolayers, SAMs) 18
2.3.5 Polyvalency at Surfaces of Viruses, Bacteria, and SAMs 18
2.4 Theoretical Considerations in Multivalency 19
2.4.1 Survey of Thermodynamics 19
2.4.2 Additivity and Multivalency 19
2.4.3 Avidity and Effective Concentration ($C_{\text{eff}}$) 22
2.4.4 Cooperativity is Distinct from Multivalency 24
2.4.5 Conformational Entropy of the Linker between Ligands 25
2.4.6 Enthalpy/Entropy Compensation Reduces the Benefit of Multivalency 26
2.5 Representative Experimental Studies 26
2.5.1 Experimental Techniques Used to Examine Multivalent Systems 26
2.5.1.1 Isothermal Titration Calorimetry 26
2.5.1.2 Surface Plasmon Resonance Spectroscopy 27
2.5.1.3 Surface Assays Using Purified Components (Cell-free Assays) 27
2.5.1.4 Cell-based Surface Assays 27
2.5.2 Examination of Experimental Studies in the Context of Theory 28
2.5.2.1 Trivalency in Structurally Simple Systems 28
2.5.2.2 Cooperativity (and the Role of Enthalpy) in the “Chelate Effect“ 29
2.5.2.3 Oligovalency in the Design of Inhibitors of Toxins 29
2.5.2.4 Bivalency in Solution and at Well Defined Surfaces (SAMs) 30
2.5.2.5 Polyvalency at Surfaces (Viruses, Bacteria, and SAMs) 31
2.6 Design Rules for Multivalent Ligands 32
2.6.1 When Will Multivalency Be a Successful Strategy to Design Tight-binding Ligands? 32
2.6.2 Choice of Scaffold for Multivalent Ligands 33
2.6.2.1 Scaffolds for Oligovalent Ligands 33
2.6.2.2 Scaffolds for Polyvalent Ligands 35
2.6.3 Choice of Linker for Multivalent Ligands 36
2.6.3.1 Rigid Linkers Represent a Simple Approach to Optimize Affinity 36
2.6.3.2 Flexible Linkers Represent an Alternative Approach to Rigid Linkers to Optimize Affinity 37
2.6.4 Strategy for the Synthesis of Multivalent Ligands 37
2.6.4.1 Polyvalent Ligands: Polymerization of Ligand Monomers 38
2.6.4.2 Polyvalent Ligands: Functionalization with Ligands after Polymerization 38
2.7 Extensions of Multivalency to Lead Discovery 39
2.7.1 Hetero-oligovalency Is a Broadly Applicable Concept in Ligand Design 39
2.7.2 Dendrimers Present Opportunities for Multivalent Presentation of Ligands 40
2.7.3 Bivalency in the Immune System 40
2.7.4 Polymers Could Be the Most Broadly Applicable Multivalent Ligands 42
2.8 Challenges and Unsolved Problems in Multivalency 44
2.9 Conclusions 44
Acknowledgments 45
References 45
6 Structural Fragments in Marketed Oral Drugs 113
Michal Vieth and Miles Siegel
6.1 Introduction 113
6.2 Historical Look at the Analysis of Structural Fragments of Drugs 113
6.3 Methodology Used in this Analysis 115
6.4 Analysis of Similarities of Different Drug Data Sets Based on the Fragment Frequencies 118
6.5 Conclusions 123
Acknowledgments 124
References 124

7 Fragment Docking to Proteins with the Multi-copy Simultaneous Search Methodology 125
Collin M. Stultz and Martin Karplus
7.1 Introduction 125
7.2 The MCSS Method 125
7.2.1 MCSS Minimizations 126
7.2.2 Choice of Functional Groups 126
7.2.3 Evaluating MCSS Minima 127
7.3 MCSS in Practice: Functionality Maps of Endothiapepsin 132
7.4 Comparison with GRID 135
7.5 Comparison with Experiment 137
7.6 Ligand Design with MCSS 138
7.6.1 Designing Peptide-based Ligands to Ras 138
7.6.2 Designing Non-peptide Based Ligands to Cytochrome P450 140
7.6.3 Designing Targeted Libraries with MCSS 140
7.7 Protein Flexibility and MCSS 141
7.8 Conclusion 143
Acknowledgments 144
References 144

Part 3: Experimental Techniques and Applications

8 NMR-guided Fragment Assembly 149
Daniel S. Sem
8.1 Historical Developments Leading to NMR-based Fragment Assembly 149
8.2 Theoretical Foundation for the Linking Effect 150
8.3 NMR-based Identification of Fragments that Bind Proteins 152
8.3.1 Fragment Library Design Considerations 152
8.3.2 The “SHAPES” NMR Fragment Library  154
8.3.3 The “SAR by NMR” Fragment Library  156
8.3.4 Fragment-based Classification of protein Targets  160
8.4 NMR-based Screening for Fragment Binding  163
8.4.1 Ligand-based Methods  163
8.4.2 Protein-based Methods  165
8.4.3 High-throughput Screening: Traditional and TINS  167
8.5 NMR-guided Fragment Assembly  167
8.5.1 SAR by NMR  167
8.5.2 SHAPES  169
8.5.3 Second-site Binding Using Paramagnetic Probes  169
8.5.4 NMR-based Docking  170
8.6 Combinatorial NMR-based Fragment Assembly  171
8.6.1 NMR SOLVE  171
8.6.2 NMR ACE  173
8.7 Summary and Future Prospects  176
References  177

9  SAR by NMR: An Analysis of Potency Gains Realized Through Fragment-linking and Fragment-elaboration Strategies for Lead Generation  181
Philip J. Hajduk, Jeffrey R. Huth, and Chaohong Sun
9.1 Introduction  181
9.2 SAR by NMR  182
9.3 Energetic Analysis of Fragment Linking Strategies  183
9.4 Fragment Elaboration  187
9.5 Energetic Analysis of Fragment Elaboration Strategies  188
9.6 Summary  190
References  191

10 Pyramid: An Integrated Platform for Fragment-based Drug Discovery  193
Thomas G. Davies, Rob L. M. van Montfort, Glyn Williams, and Harren Jhoti
10.1 Introduction  193
10.2 The Pyramid Process  194
10.2.1 Introduction  194
10.2.2 Fragment Libraries  195
10.2.2.1 Overview  195
10.2.2.2 Physico-chemical Properties of Library Members  196
10.2.2.3 Drug Fragment Library  197
10.2.2.4 Privileged Fragment Library  197
10.2.2.5 Targeted Libraries and Virtual Screening  197
10.2.2.6 Quality Control of Libraries  201
10.2.3 Fragment Screening  201
10.2.4 X-ray Data Collection  202

Jeff Blaney, Vicki Nienaber, and Stephen K. Burley

11.1 Introduction 215

11.2 Overview of the SGX Structure-driven Fragment-based Lead Discovery Process 217

11.3 Fragment Library Design for Crystallographic Screening 218

11.3.1 Considerations for Selecting Fragments 218

11.3.2 SGX Fragment Screening Library Selection Criteria 219

11.3.3 SGX Fragment Screening Library Properties 220

11.3.4 SGX Fragment Screening Library Diversity: Theoretical and Experimental Analyses 220

11.4 Crystallographic Screening of the SGX Fragment Library 221

11.4.1 Overview of Crystallographic Screening 222

11.4.2 Obtaining the Initial Target Protein Structure 224

11.4.3 Enabling Targets for Crystallographic Screening 225

11.4.4 Fragment Library Screening at SGX-CAT 225

11.4.5 Analysis of Fragment Screening Results 226

11.4.6 Factor VIIa Case Study of SGX Fragment Library Screening 228

11.5 Complementary Biochemical Screening of the SGX Fragment Library 230

11.6 Importance of Combining Crystallographic and Biochemical Fragment Screening 232

11.7 Selecting Fragments Hits for Chemical Elaboration 233

11.8 Fragment Optimization 234

11.8.1 Spleen Tyrosine Kinase Case Study 234

11.8.2 Fragment Optimization Overview 240

11.8.3 Linear Library Optimization 241

11.8.4 Combinatorial Library Optimization 242

11.9 Discussion and Conclusions 243

11.10 Postscript: SGX Oncology Lead Generation Program 245

References 245
12 Synergistic Use of Protein Crystallography and Solution-phase NMR Spectroscopy in Structure-based Drug Design: Strategies and Tactics 249
Cele Abad-Zapatero, Geoffrey F. Stamper, and Vincent S. Stoll

12.1 Introduction 249
12.2 Case 1: Human Protein Tyrosine Phosphatase 252
12.2.1 Designing and Synthesizing Dual-site Inhibitors 252
12.2.1.1 The Target 252
12.2.1.2 Initial Leads 252
12.2.1.3 Extension of the Initial Fragment 254
12.2.1.4 Discovery and Incorporation of the Second Fragment 256
12.2.1.5 The Search for Potency and Selectivity 257
12.2.2 Finding More “Drug-like” Molecules 258
12.2.2.1 Decreasing Polar Surface Area on Site 2 258
12.2.2.2 Monoacid Replacements on Site 1 258
12.2.2.3 Core Replacement 259
12.3 Case 2: MurF 261
12.3.1 Pre-filtering by Solution-phase NMR for Rapid Co-crystal Structure Determinations 261
12.3.1.1 The Target 261
12.3.1.2 Triage of Initial Leads 261
12.3.1.3 Solution-phase NMR as a Pre-filter for Co-crystallization Trials 262
12.4 Conclusion 263
Acknowledgments 264
References 264

13 Ligand SAR Using Electrospray Ionization Mass Spectrometry 267
Richard H. Griffey and Eric E. Swayze

13.1 Introduction 267
13.2 ESI-MS of Protein and RNA Targets 268
13.2.1 ESI-MS Data 268
13.2.2 Signal Abundances 268
13.3 Ligands Selected Using Affinity Chromatography 271
13.3.1 Antibiotics Binding Bacterial Cell Wall Peptides 272
13.3.2 Kinases and GPCRs 272
13.3.3 Src Homology 2 Domain Screening 273
13.3.4 Other Systems 274
13.4 Direct Observation of Ligand–Target Complexes 275
13.4.1 Observation of Enzyme–Ligand Transition State Complexes 276
13.4.2 Ligands Bound to Structured RNA 276
13.4.3 ESI-MS for Linking Low-affinity Ligands 277
13.5 Unique Features of ESI-MS Information for Designing Ligands 282
References 282
14  Tethering  285
   Daniel A. Erlanson, Marcus D. Ballinger, and James A. Wells
14.1  Introduction  285
14.2  Energetics of Fragment Selection in Tethering  286
14.3  Practical Considerations  289
14.4  Finding Fragments  289
14.4.1  Thymidylate Synthase: Proof of Principle  289
14.4.2  Protein Tyrosine Phosphatase 1B: Finding Fragments in a Fragile, Narrow Site  292
14.5  Linking Fragments  293
14.5.1  Interleukin-2: Use of Tethering to Discover Small Molecules that Bind to a Protein–Protein Interface  293
14.5.2  Caspase-3: Finding and Combining Fragments in One Step  296
14.5.3  Caspase-1  299
14.6  Beyond Traditional Fragment Discovery  300
14.6.1  Caspase-3: Use of Tethering to Identify and Probe an Allosteric Site  300
14.6.2  GPCRs: Use of Tethering to Localize Hits and Confirm Proposed Binding Models  303
14.7  Related Approaches  306
14.7.1  Disulfide Formation  306
14.7.2  Imine Formation  307
14.7.3  Metal-mediated  307
14.8  Conclusions  308
Acknowledgments  308
References  308

Part 4: Emerging Technologies in Chemistry

15  Click Chemistry for Drug Discovery  313
   Stefanie Röper and Hartmut C. Kolb
15.1  Introduction  313
15.2  Click Chemistry Reactions  314
15.3  Click Chemistry in Drug Discovery  316
15.3.1  Lead Discovery Libraries  316
15.3.2  Natural Products Derivatives and the Search for New Antibiotics  317
15.3.3  Synthesis of Neoglycoconjugates  320
15.3.4  HIV Protease Inhibitors  321
15.3.5  Synthesis of Fucosyltranferase Inhibitor  323
15.3.6  Glycoarrays  324
15.4  In Situ Click Chemistry  325
15.4.1  Discovery of Highly Potent AChE by In Situ Click Chemistry  325
15.5  Bioconjugation Through Click Chemistry  328
15.5.1  Tagging of Live Organisms and Proteins  328
15.5.2 Activity-based Protein Profiling 330
15.5.3 Labeling of DNA 332
15.5.4 Artificial Receptors 333
15.6 Conclusion 334
References 335

16 Dynamic Combinatorial Diversity in Drug Discovery 341
Matthias Hochgürtel and Jean-Marie Lehn
16.1 Introduction 341
16.2 Dynamic Combinatorial Chemistry – The Principle 342
16.3 Generation of Diversity: DCC Reactions and Building Blocks 343
16.4 DCC Methodologies 346
16.5 Application of DCC to Biological Systems 347
16.5.1 Enzymes as Targets 349
16.5.2 Receptor Proteins as Targets 355
16.5.3 Nucleotides as Targets 357
16.6 Summary and Outlook 359
References 361

Index 365