

Contents

Volume 1

Preface XXVII

List of Contributors XXXI

| | | |
|----------|---|-----------|
| 1 | Guidelines for the Functional Analysis of Engineered and Mutant Enzymes | 1 |
| | <i>Dale E. Edmondson and Giovanni Gadda</i> | |
| 1.1 | Introduction | 1 |
| 1.2 | Steady-State Kinetics | 2 |
| 1.3 | Enzyme Assays and the Acquisition of Initial Velocity Data | 3 |
| 1.3.1 | Biological Sample Appropriate for Assay | 3 |
| 1.3.2 | Enzymatic Assays | 4 |
| 1.3.3 | Analysis of Initial Rate Data | 6 |
| 1.3.4 | Determination of Functional Catalytic Site Concentrations | 8 |
| 1.4 | Steady-State Kinetic Parameters and Their Interpretation | 8 |
| 1.4.1 | pH-Dependence of Steady-State Kinetic Parameters | 11 |
| 1.4.2 | Analysis of Two-Substrate Enzymes | 11 |
| 1.5 | Concluding Remarks | 12 |
| | References | 12 |
| 2 | Engineering Enantioselectivity in Enzyme-Catalyzed Reactions | 15 |
| | <i>Romas Kazlauskas</i> | |
| 2.1 | Introduction | 15 |
| 2.2 | Molecular Basis for Enantioselectivity | 18 |
| 2.2.1 | Enzymes Stabilize Transition States for Fast-Reacting Enantiomers Better than Slow-Reacting Enantiomers | 18 |
| 2.2.2 | The Slow-Reacting Enantiomer Fits by Exchanging Two Substituents | 18 |
| 2.2.3 | The Slow Enantiomer Fits by an Umbrella-Like Inversion | 19 |
| 2.3 | Qualitative Predictions of Enantioselectivity | 23 |

| | | |
|-------|--|----|
| 2.3.1 | Comparing Substrate Structures Leads to Empirical Rules and Box Models | 23 |
| 2.3.2 | Computer Modeling Based on X-Ray Structures of Enzymes | 25 |
| 2.3.3 | What Is Missing from Current Computer Modeling? | 26 |
| 2.4 | Protein Engineering to Increase or Reverse Enantioselectivity | 30 |
| 2.4.1 | Mutations Closer to the Active Site Increase Enantioselectivity More Effectively than Mutations Far from the Active Site | 30 |
| 2.4.2 | Reversing Enantioselectivity by Exchanging Locations of Binding Sites or a Catalytic Group | 36 |
| 2.5 | Concluding Remarks | 40 |
| | References | 41 |

3 Mechanism and Catalytic Promiscuity: Emerging Mechanistic Principles for Identification and Manipulation of Catalytically Promiscuous Enzymes 47

Stefanie Jonas and Florian Hollfelder

| | | |
|-------|---|----|
| 3.1 | Introduction | 47 |
| 3.2 | Calculation of Rate Accelerations | 52 |
| 3.3 | Catalytic Features and Their Propensity for Promiscuity | 55 |
| 3.3.1 | Metal Ions | 55 |
| 3.3.2 | Recognition of Transition State Charges: Analysis of the Nature of the Transition State | 61 |
| 3.3.3 | Catalytic Dyads and Triads | 63 |
| 3.3.4 | General Acid/Base Catalysts in Promiscuous Functional Motifs in Catalytic Superfamilies | 64 |
| 3.4 | Steric Effects and Structural Constriction in the Active Site: <i>Product</i> Promiscuity | 67 |
| 3.5 | Medium Effects in Enzyme Active Sites | 70 |
| 3.6 | Conclusions | 71 |
| | References | 72 |

4 Φ -Value Analysis of Protein Folding Transition States 81

Neil Ferguson and Alan R. Fersht

| | | |
|---------|---|----|
| 4.1 | Introduction | 81 |
| 4.2 | Theoretical Principles of Protein Engineering | 82 |
| 4.2.1 | Overview | 82 |
| 4.2.2 | Basic Concepts | 83 |
| 4.2.3 | Theory of Φ -Value Analysis | 87 |
| 4.2.4 | Relationship between Φ and Leffler α | 90 |
| 4.2.5 | Linear Free-Energy Relationships and Denaturant Concentration | 93 |
| 4.3 | Guidelines for the Determination of Accurate Φ -Values | 95 |
| 4.3.1 | Buffer Preparation and Selection | 96 |
| 4.3.2 | Optimization of Experimental Conditions | 97 |
| 4.3.3 | Equilibrium Denaturation Experiments | 99 |
| 4.3.3.1 | Practical Considerations | 99 |

| | | |
|----------|---|------------|
| 4.3.3.2 | Curve-Fitting | 103 |
| 4.3.4 | Kinetic Measurements | 105 |
| 4.3.4.1 | Practical Considerations | 107 |
| 4.3.4.2 | Curve Fitting | 110 |
| 4.3.4.3 | Error Analysis for Chevron Plots | 113 |
| 4.4 | Conclusions | 115 |
| | Acknowledgments | 116 |
| | References | 116 |
| 5 | Protein Folding and Solubility: Pathways and High-Throughput Assays | 121 |
| | <i>Adam C. Fisher, Thomas J. Mansell, and Matthew P. DeLisa</i> | |
| 5.1 | Introduction | 121 |
| 5.2 | Biosynthesis of Natural Proteins in Bacteria | 122 |
| 5.2.1 | Recombinant Protein Folding | 122 |
| 5.2.2 | Protein Misfolding and Inclusion Body Formation | 123 |
| 5.2.3 | Proteolysis | 124 |
| 5.2.4 | Cytoplasmic Chaperones | 124 |
| 5.2.5 | Export Pathways | 125 |
| 5.3 | Biosynthesis of <i>de novo</i> -Designed Proteins in Bacteria | 126 |
| 5.4 | Combinatorial Strategies for Assaying Protein Folding in Bacteria | 126 |
| 5.4.1 | Initial Protein-Folding Studies | 128 |
| 5.4.2 | Protein Chimeras | 128 |
| 5.4.3 | Split Proteins | 129 |
| 5.4.4 | Genetic Response | 130 |
| 5.4.5 | Cellular Quality Control Systems | 130 |
| 5.5 | Structural Genomics | 131 |
| 5.6 | Protein-Misfolding Diseases | 132 |
| 5.7 | Future Directions | 135 |
| 5.7.1 | Folding versus Solubility | 137 |
| | References | 138 |
| 6 | Protein Dynamics and the Evolution of Novel Protein Function | 147 |
| | <i>Jörg Zimmermann, Megan C. Thielges, Wayne Yu and Floyd E. Romesberg</i> | |
| 6.1 | Introduction | 147 |
| 6.2 | Physical Background | 149 |
| 6.2.1 | Flexibility, Conformational Heterogeneity and Time Scales of Protein Dynamics | 149 |
| 6.2.2 | Protein Dynamics and Thermodynamics of Molecular Recognition | 151 |
| 6.3 | Experimental Studies of Protein Dynamics | 153 |
| 6.3.1 | NMR Relaxation Experiments | 153 |
| 6.3.2 | Ultrafast Laser Spectroscopy | 154 |
| 6.4 | Experimental Techniques | 158 |

- 6.4.1 Time-Correlation Function and the Spectral Density of Protein Motions 158
- 6.4.2 NMR Relaxation Techniques to Determine $\rho(\omega)$ 160
- 6.4.3 Ultrafast Laser Spectroscopy to Determine $C(t)$ and $\rho(\omega)$ 160
- 6.4.4 Additional Approaches to the Characterization of Protein Dynamics 162
- 6.4.5 Chromophores to Probe Protein Dynamics 164
- 6.5 Case Study: Protein Dynamics and the Evolution of Molecular Recognition within the Immune System 165
- 6.6 Implications for Protein Engineering 172
- References 173

7 Gaining Insight into Enzyme Function through Correlation with Protein Motions 187

Nicolas Doucet and Joelle N. Pelletier

- 7.1 Introduction 187
 - 7.1.1 Enzyme Catalysis—the Origin of Rate Acceleration 187
 - 7.1.2 Proteins Are Intrinsically Dynamic Molecules 188
 - 7.1.3 Are Protein Motions Essential in Promoting the Catalytic Step of Enzyme Reactions? 190
- 7.2 Experimental Investigation of Enzyme Dynamics during Catalysis 191
 - 7.2.1 Quantum Tunneling Revealed by Unusually Large Kinetic Isotope Effects (KIEs): Are Enzyme Dynamics Involved? 191
 - 7.2.1.1 Varying Atomic Mass Can Alter the Rate of Proton Transfer 192
 - 7.2.1.2 KIEs Reveal Quantum Tunneling 192
 - 7.2.1.3 Quantum Tunneling and Protein Dynamics 192
 - 7.2.2 Nuclear Magnetic Resonance: Experimental Observation of Protein Dynamics over a Broad Range of Time Scales 193
 - 7.2.2.1 Extracting Information on Protein Dynamics by NMR 194
 - 7.2.2.2 NMR Dynamics of Enzymes 194
 - 7.2.3 Crystallographic Evidence of Motions in Enzymes 197
 - 7.2.3.1 Time-Resolved X-Ray Crystallography 197
 - 7.2.3.2 Motional Behavior in the Course of Enzyme Action 198
 - 7.2.4 Computational Methods 199
 - 7.2.4.1 Molecular Dynamics Simulations: Computational Models of Protein Motions 199
 - 7.2.4.2 Combining Quantum Mechanics with Molecular Mechanics: QM/MM 200
- 7.3 Future Challenges 201
 - 7.3.1 Promising New Methodologies for the Study of Enzyme Dynamics 201
 - 7.3.2 NMR: Improving Methodologies 202
 - 7.3.3 Kinetic Crystallography: Snapshots of a Protein in Various States 203

| | | |
|----------|---|------------|
| 7.3.4 | Computational Advances | 204 |
| | Acknowledgments | 205 |
| | References | 205 |
| 8 | Structural Frameworks Suitable for Engineering | 213 |
| | <i>Birte Höcker</i> | |
| 8.1 | Introduction | 213 |
| 8.2 | Choice of Protein Scaffold in Engineering: General Considerations | 214 |
| 8.3 | Examples of Engineered Structural Frameworks in Natural Evolution | 215 |
| 8.3.1 | The $(\beta\alpha)_8$ -Barrel Fold: A Natural Framework for Catalytic Function | 216 |
| 8.3.3.1 | Features of the $(\beta\alpha)_8$ -Barrel Fold | 217 |
| 8.3.1.2 | Engineering Experiments with $(\beta\alpha)_8$ -Barrel Proteins | 218 |
| 8.3.2 | Periplasmic Binding Proteins: Using the Flexible Hinge | 220 |
| 8.3.2.1 | Features of the PBP Fold | 221 |
| 8.3.2.2 | Biosensors, Switches and Computational Design | 221 |
| 8.3.3 | Repeat Proteins: Binding Large Molecules | 223 |
| 8.3.3.1 | Features of the Repeat Folds | 224 |
| 8.3.3.2 | Engineering Approaches with Repeat Folds | 225 |
| 8.4 | Summary | 226 |
| | References | 227 |
| 9 | Microbes and Enzymes: Recent Trends and New Directions to Expand Protein Space | 233 |
| | <i>Ana Beloqui, Miren Zumárraga, Miguel Alcalde, Peter N. Golyshin, and Manuel Ferrer</i> | |
| 9.1 | Introduction | 233 |
| 9.2 | Protein Complexity of Microbial Communities through Metagenomics | 233 |
| 9.3 | Important Methodological Developments in Metagenomics | 236 |
| 9.3.1 | DNA Extraction Methodologies | 236 |
| 9.3.1.1 | Separation of Cellular Biomass from a Soil Homogenate via a Nycodenz Gradient | 238 |
| 9.3.1.2 | Isolation of High-Quality DNA by Phenol:Chloroform Method Followed by DNA Cleaning | 238 |
| 9.3.1.3 | Isolation of High-Quality DNA with Commercial Kits | 239 |
| 9.3.2 | Functional Expression in Heterologous Hosts | 241 |
| 9.3.2.1 | Materials | 242 |
| 9.3.2.2 | Method for DNA Separation | 242 |
| 9.3.2.3 | Method for DNA Fragmentation | 243 |
| 9.3.3 | Amplification and Subtraction of Whole Genomes in Low-Biomass Samples | 243 |

| | | |
|-------|--|-----|
| 9.3.4 | Phylogenetic Affiliation of Metagenomic Fragments | 244 |
| 9.4 | Metagenomic Analysis of Whole-Metagenome Sequences: Shotgun Sequencing and Pyrosequencing | 245 |
| 9.5 | Bottlenecks in the Discovery of 'Natural' Proteins | 246 |
| 9.5.1 | PCR-Based Approach | 246 |
| 9.5.2 | Methods of Nucleic Acid Capture | 248 |
| 9.5.3 | Indirect Methods by Using Genetic Traps and Quorum-Sensing Promoters | 249 |
| 9.5.4 | Mutational Screening Methods | 249 |
| 9.5.5 | Supplementation Methods | 249 |
| 9.5.6 | Functional Screening Methods | 250 |
| 9.6 | Conclusions to Metagenomics for Gene Discovery: The Limits of 'Natural' Protein Diversity | 253 |
| 9.7 | Directed Molecular Evolution for Creating 'Artificial' Protein Diversity | 254 |
| 9.8 | Generation of Diversity <i>in vitro</i> | 256 |
| 9.8.1 | Random Mutagenesis | 256 |
| 9.8.2 | Methods of DNA Recombination | 258 |
| 9.8.3 | <i>In vivo</i> Methods | 258 |
| 9.8.4 | <i>In vivo</i> Methods Using <i>S. cerevisiae</i> as a Tool for the Generation of Diversity | 259 |
| 9.9 | Semi-Rational Approaches: Saturation Mutagenesis | 260 |
| 9.10 | The Development of Efficient Screening Methods | 261 |
| 9.11 | Metagenomic DNA Shuffling: Increasing Protein Complexity by Combining 'Natural' and 'Artificial' Diversity | 262 |
| | Acknowledgments | 263 |
| | References | 264 |

10 Inteins in Protein Engineering 271

Alison R. Gillies and David W. Wood

| | | |
|--------|---|-----|
| 10.1 | Introduction | 271 |
| 10.1.1 | Inteins | 271 |
| 10.1.2 | Origin and Evolution | 272 |
| 10.1.3 | Structure | 273 |
| 10.1.4 | Splicing Mechanism | 274 |
| 10.1.5 | Overview of Applications in Protein Engineering | 276 |
| 10.2 | Expressed Protein Ligation | 277 |
| 10.2.1 | EPL Methods | 277 |
| 10.2.2 | Applications of EPL | 279 |
| 10.3 | Protein <i>trans</i> -Splicing | 280 |
| 10.3.1 | PTS Methods | 280 |
| 10.3.2 | Applications of PTS | 282 |
| 10.4 | Cyclization of Proteins | 282 |
| 10.4.1 | Cyclization Methods | 284 |
| 10.4.2 | Applications of Cyclization | 285 |

| | | |
|-----------|--|------------|
| 10.5 | Protein <i>cis</i> -Splicing and Cleaving | 285 |
| 10.5.1 | <i>Cis</i> -Splicing or Cleaving Methods | 285 |
| 10.5.2 | Applications of <i>cis</i> -Splicing or Cleaving | 286 |
| 10.6 | Potential Future Uses in Protein Engineering | 288 |
| | References | 289 |
| 11 | From Prospecting to Product—Industrial Metagenomics Is Coming of Age | 295 |
| | <i>Jürgen Eck, Esther Gabor, Klaus Liebeton, Guido Meurer, and Frank Niehaus</i> | |
| 11.1 | Prospecting for Novel Templates | 295 |
| 11.1.1 | Metagenome—a Definition | 295 |
| 11.1.2 | Microorganisms as the Predominant Life-Form | 296 |
| 11.1.3 | Microbial Diversity and the Problem of Cultivation | 296 |
| 11.1.4 | Molecular Genetic Analysis of Diversity | 297 |
| 11.2 | Sample Generation: Access to the Metagenome | 298 |
| 11.2.1 | Preparation of Metagenomic DNA | 298 |
| 11.2.2 | Purification and Amplification of Metagenomic DNA | 299 |
| 11.2.3 | Construction of Metagenomic Gene Libraries | 301 |
| 11.2.4 | Increasing Hit Rates of Target Enzymes | 303 |
| 11.2.5 | Recovering Enzyme-Encoding Genes from the Metagenome | 303 |
| 11.3 | Sequence-Based Screening | 307 |
| 11.3.1 | Screening of Metagenome Libraries | 307 |
| 11.3.2 | Direct Access to Metagenome Sequence Information | 308 |
| 11.4 | Activity-Based Screening | 309 |
| 11.4.1 | Screening of Metagenome Expression Libraries | 309 |
| 11.4.2 | Heterologous Gene Expression: Transcription and Translation | 310 |
| 11.4.3 | Codon Usage | 311 |
| 11.4.4 | Alternative Expression Hosts | 312 |
| 11.4.5 | Assay Systems | 313 |
| 11.5 | Metagenomics—the Industrial Perspective | 315 |
| | References | 316 |
| 12 | Computational Protein Design | 325 |
| | <i>Jeffery G. Saven</i> | |
| 12.1 | Introduction | 325 |
| 12.2 | Methods of Computational Protein Design | 327 |
| 12.2.1 | Target Structure | 327 |
| 12.2.2 | Degrees of Freedom | 327 |
| 12.2.3 | Energy Function | 328 |
| 12.2.4 | Solvation and Patterning | 328 |
| 12.2.5 | Search Methods | 329 |
| 12.3 | Computationally Designed Proteins | 329 |
| 12.3.1 | Protein Re-Engineering | 330 |
| 12.3.2 | De novo-Designed Proteins | 333 |

| | | |
|-----------|--|------------|
| 12.3.2.1 | Structure | 333 |
| 12.3.2.2 | Metal-Binding Sites | 333 |
| 12.3.2.3 | Cofactors | 334 |
| 12.3.2.4 | Protein Folding | 335 |
| 12.3.2.5 | Membrane Proteins | 336 |
| 12.3.2.6 | Enzymatic Catalysis | 336 |
| 12.4 | Outlook | 337 |
| | Acknowledgments | 338 |
| | References | 338 |
| 13 | Assessing and Exploiting the Persistence of Substrate Ambiguity in Modern Protein Catalysts | 343 |
| | <i>Kevin K. Desai and Brian G. Miller</i> | |
| 13.1 | Quantitative Description of Enzyme Specificity | 343 |
| 13.2 | Models of Enzyme Specificity | 345 |
| 13.3 | Advantages and Disadvantages of Specificity | 346 |
| 13.4 | Substrate Ambiguity as a Mechanism for Elaborated Metabolic Potential | 347 |
| 13.5 | Experimental Approaches to Detect Ambiguity | 348 |
| 13.5.1 | Whole Cell Mutagenesis and Selection | 349 |
| 13.5.2 | Phenotypic Screening | 350 |
| 13.5.3 | Overexpression Libraries | 351 |
| 13.5.3.1 | Purification of Genomic DNA | 351 |
| 13.5.3.2 | Generating Genomic DNA Fragments | 353 |
| 13.5.3.3 | Preparation of Vector DNA | 353 |
| 13.5.3.4 | Ligation and Transformation of Libraries | 354 |
| 13.6 | General Comments on Overexpression Libraries and Genetic Selections | 354 |
| 13.7 | Challenges and Prospects for the Future | 356 |
| 13.7.1 | Functional Genomics | 356 |
| 13.7.2 | Metagenomic Libraries | 357 |
| 13.7.3 | Universal Genetic Selection Systems | 358 |
| | References | 359 |
| 14 | Designing Programmable Protein Switches | 363 |
| | <i>Martin Sagermann</i> | |
| 14.1 | Introduction | 363 |
| 14.2 | Engineering Allostery | 365 |
| 14.3 | A Fundamental Experimental Challenge | 365 |
| 14.3.1 | Engineering of Side-Chain Allostery | 366 |
| 14.3.2 | Secondary Structure Transitions | 367 |
| 14.3.3 | Designing Proteins that Adopt Different Folds with the Same Sequence | 368 |
| 14.3.4 | Insertion of Conformational Switches | 369 |
| 14.4 | A Different Approach: Creation of Internal Sequence Repeats | 369 |

| | | |
|--------|---|-----|
| 14.4.1 | Experimental Details | 370 |
| 14.4.2 | Switching Conformations Through Secondary Structure Transitions | 371 |
| 14.4.3 | Duplication and Switching of β -Strands | 373 |
| 14.4.4 | Duplication of an α -Helix | 377 |
| 14.4.5 | Circular Permutations | 381 |
| 14.5 | Engineering a Conundrum | 383 |
| 14.6 | Advantages of Sequence Duplications, and Possible Future Applications | 384 |
| | Acknowledgments | 385 |
| | References | 385 |

15 The Cyclization of Peptides and Proteins with Inteins 391

Blaise R. Boles and Alexander R. Horswill

| | | |
|--------|--|-----|
| 15.1 | Introduction | 391 |
| 15.2 | Protein Cyclization | 393 |
| 15.2.1 | <i>In vitro</i> Protein Cyclization | 393 |
| 15.2.2 | <i>In vivo</i> Protein Cyclization | 395 |
| 15.3 | Cyclization of Peptides | 396 |
| 15.3.1 | Intein Generation of <i>in vivo</i> Cyclic Peptide Libraries | 398 |
| 15.3.2 | Applications of <i>in vivo</i> Cyclic Peptide Libraries | 398 |
| 15.3.3 | Other Applications of Intein-Catalyzed Cyclization | 400 |
| 15.3.4 | Future Directions | 402 |
| 15.4 | Conclusions | 403 |
| | References | 403 |

Volume 2

16 A Method for Rapid Directed Evolution 409

Manfred T. Reetz

| | | |
|--------|---|-----|
| 16.1 | Introduction | 409 |
| 16.2 | Focused Libraries Generated by Saturation Mutagenesis | 414 |
| 16.3 | Iterative Saturation Mutagenesis | 416 |
| 16.3.1 | General Concept | 416 |
| 16.3.2 | Combinatorial Active-Site Saturation Test (CAST) as a Means to Control Substrate Acceptance and/or Enantioselectivity | 418 |
| 16.3.3 | B-Factor Iterative Test (B-FIT) as a Means to Increase Thermostability | 425 |
| 16.3.4 | Practical Hints for Applying ISM | 430 |
| 16.4 | Conclusions | 430 |
| | References | 431 |

| | | |
|-----------|--|------------|
| 17 | Evolution of Enantioselective <i>Bacillus subtilis</i> Lipase | 441 |
| | <i>Thorsten Eggert, Susanne A. Funke, Jennifer N. Andexer, Manfred T. Reetz and Karl-Erich Jaeger</i> | |
| 17.1 | Introduction | 441 |
| 17.2 | Directed Evolution of Enantioselective Lipase from <i>Bacillus subtilis</i> | 444 |
| 17.3 | Directed Evolution by Error-Prone PCR | 445 |
| 17.4 | Complete Site-Saturation Mutagenesis | 446 |
| 17.5 | Conclusions | 448 |
| | References | 449 |
| | | |
| 18 | Circular Permutation of Proteins | 453 |
| | <i>Glenna E. Meister, Manu Kanwar, and Marc Ostermeier</i> | |
| 18.1 | Introduction | 453 |
| 18.2 | Evolution of Circular Permutations in Nature | 454 |
| 18.2.1 | Naturally Occurring Circular Permutations | 454 |
| 18.2.2 | Identification of Natural Circular Permutations | 455 |
| 18.2.3 | Mechanisms of Circular Permutation | 457 |
| 18.3 | Artificial Circular Permutations | 459 |
| 18.3.1 | Early Studies | 459 |
| 18.3.2 | Systematic and Random Circular Permutation | 460 |
| 18.3.3 | Protein Folding and Stability | 462 |
| 18.4 | Circular Permutation and Protein Engineering | 463 |
| 18.4.1 | Alteration of the Spatial Arrangement of Protein Fusions | 463 |
| 18.4.2 | Oligomeric State Modification | 464 |
| 18.4.3 | Improvement of Function | 465 |
| 18.4.4 | Creation of Protein Switches | 466 |
| 18.4.5 | Protein Crystallization | 467 |
| 18.5 | Perspective | 468 |
| | Acknowledgments | 468 |
| | References | 468 |
| | | |
| 19 | Incorporating Synthetic Oligonucleotides via Gene Reassembly (ISOR): A Versatile Tool for Generating Targeted Libraries | 473 |
| | <i>Asael Herman and Dan S. Tawfik</i> | |
| 19.1 | Introduction | 473 |
| 19.1.1 | Background | 473 |
| 19.1.2 | Overview of the Method | 474 |
| 19.1.3 | Applications | 475 |
| 19.2 | Materials | 475 |
| 19.2.1 | DNaseI Digestion | 475 |
| 19.2.2 | Assembly | 476 |
| 19.2.3 | Magnetic Separation and Product Amplification | 476 |
| 19.3 | Methods | 476 |
| 19.3.1 | DNaseI Digestion | 476 |

| | | |
|-----------|--|------------|
| 19.3.2 | Assembly | 477 |
| 19.3.3 | Magnetic Separation and Product Amplification | 477 |
| 19.4 | Notes | 478 |
| | Acknowledgments | 479 |
| | References | 479 |
| 20 | Protein Engineering by Structure-Guided SCHEMA Recombination | 481 |
| | <i>Gloria Saab-Rincon, Yougen Li, Michelle Meyer, Martina Carbone, Marco Landwehr, and Frances H. Arnold</i> | |
| 20.1 | Introduction | 481 |
| 20.1.1 | SCHEMA Recombination of Proteins: Theoretical Framework | 481 |
| 20.1.2 | Comparison of SCHEMA with Other Guided-Recombination Methods | 483 |
| 20.1.3 | Practical Guidelines for SCHEMA Recombination | 485 |
| 20.2 | Examples of Chimeric Libraries Designed Using the SCHEMA Algorithm | 485 |
| 20.2.1 | SCHEMA Recombination of β -Lactamases | 485 |
| 20.2.2 | SCHEMA-Guided Recombination of Cytochrome P450 Heme Domains | 486 |
| 20.3 | Conclusions | 490 |
| | References | 491 |
| 21 | Chimeragenesis in Protein Engineering | 493 |
| | <i>Manuela Trani and Stefan Lutz</i> | |
| 21.1 | Introduction | 493 |
| 21.1.1 | Homology-Independent <i>in vitro</i> Recombination (Chimeragenesis) | 494 |
| 21.1.1.1 | Homology-Independent Random Gene Fusion | 494 |
| 21.1.1.2 | Homology-Independent Recombination with Multiple Crossovers | 496 |
| 21.1.2 | Predictive Algorithms in Chimeragenesis | 498 |
| 21.2 | Experimental Aspects of the SCRATCHY Protocol | 499 |
| 21.2.1 | Creation of ITCHY Libraries | 499 |
| 21.2.2 | Size and Reading Frame Selection | 501 |
| 21.2.3 | Enhanced SCRATCHY via Forced Crossovers | 503 |
| 21.3 | Future Trends in Chimeragenesis | 506 |
| 21.3.1 | Combining SCRATCHY and SCHEMA | 508 |
| 21.3.2 | The Future of Chimeragenesis | 508 |
| 21.4 | Conclusions | 511 |
| | Acknowledgments | 511 |
| | References | 511 |

| | | |
|-----------|--|------------|
| 22 | Protein Generation Using a Reconstituted System | 515 |
| | <i>Bei-Wen Ying and Takuya Ueda</i> | |
| 22.1 | Introduction | 515 |
| 22.2 | The PURE System | 516 |
| 22.2.1 | Concept and Strategy | 516 |
| 22.2.2 | The Composition of PURE | 517 |
| 22.2.3 | Advantages of PURE | 517 |
| 22.2.4 | Preparation of the Components | 519 |
| 22.2.4.1 | Overexpression and Purification of Translation Factors | 519 |
| 22.2.4.2 | Preparation of Ribosomes | 520 |
| 22.2.5 | Set-Up of the Translation Reaction | 522 |
| 22.3 | Current Applications | 523 |
| 22.3.1 | Protein Generation | 523 |
| 22.3.2 | <i>In vitro</i> Selection | 528 |
| 22.3.3 | Extensive Relevance in Mechanism Studies | 529 |
| 22.4 | Prospective Research | 530 |
| 22.4.1 | Modifications and Developments | 531 |
| 22.4.2 | Artificial Cells | 531 |
| 22.4.3 | Complexity and Network | 532 |
| 22.5 | Concluding Remarks | 532 |
| | References | 533 |
| 23 | Equipping <i>in vivo</i> Selection Systems with Tunable Stringency | 537 |
| | <i>Martin Neuenschwander, Andreas C. Kleeb, Peter Kast, and Donald Hilvert</i> | |
| 23.1 | Genetic Selection in Directed Evolution Experiments | 537 |
| 23.2 | Inducible Promoters for Controlling Selection Stringency | 538 |
| 23.2.1 | Problems Associated with Commonly Used Inducible Promoter Systems | 539 |
| 23.2.2 | Engineering Graded Homogeneous Gene Expression | 540 |
| 23.2.3 | An Optimized Tetracycline-Based Promoter System for Directed Evolution | 543 |
| 23.3 | Controlling Catalyst Concentration | 545 |
| 23.3.1 | Reducing Catalyst Concentration by Switching to Weaker Promoters | 545 |
| 23.3.2 | Reducing Catalyst Concentration through Graded Transcriptional Control | 547 |
| 23.3.3 | Combining Graded Transcriptional Control and Protein Degradation | 547 |
| 23.3.4 | General Considerations | 549 |
| 23.4 | Controlling Substrate Concentrations | 550 |
| 23.4.1 | Engineering a Tunable Selection System Controlled by Substrate Concentration | 551 |
| 23.4.2 | Applications | 554 |
| 23.4.3 | Advantages of Metabolic Engineering Approaches | 555 |

| | | |
|-----------|--|------------|
| 23.5 | Perspectives | 556 |
| | References | 557 |
| 24 | Protein Engineering by Phage Display | 563 |
| | <i>Agathe Urvoas, Philippe Minard, and Patrice Soumilion</i> | |
| 24.1 | Introduction | 563 |
| 24.2 | The State of the Art | 563 |
| 24.2.1 | Engineering Protein Binders by Phage Display | 563 |
| 24.2.1.1 | Antibodies and Antibody Fragments | 563 |
| 24.2.1.2 | Alternative Scaffolds | 566 |
| 24.2.2 | Engineering Protein Stability by Phage Display | 571 |
| 24.2.3 | Engineering Enzymes by Phage Display | 573 |
| 24.2.3.1 | Engineering Allosteric Regulation | 573 |
| 24.2.3.2 | Engineering Catalytic Activity | 574 |
| 24.3 | Practical Considerations | 578 |
| 24.3.1 | Choosing a Vector | 578 |
| 24.3.2 | Phage Production | 582 |
| 24.3.3 | Phage Purification | 582 |
| 24.3.3.1 | PEG Precipitation | 583 |
| 24.3.3.2 | CsCl Equilibrium Gradient | 583 |
| 24.3.4 | Measuring Phage Titer | 583 |
| 24.3.5 | Measuring Phage Concentration | 584 |
| 24.3.6 | Evaluating the Level of Display | 584 |
| 24.3.6.1 | Western Blot | 584 |
| 24.3.6.2 | Active-Site Labeling | 584 |
| 24.3.7 | Measuring the Affinity of a Phage for a Ligand | 585 |
| 24.3.8 | Measuring the Activity of a Phage-Enzyme | 585 |
| 24.3.9 | Library Construction | 585 |
| 24.3.10 | Library Production | 586 |
| 24.3.11 | Selections | 587 |
| 24.3.11.1 | Affinity-Based Selections | 587 |
| 24.3.11.2 | Activity-Based Selections of Phage-Enzymes | 588 |
| 24.3.12 | Troubleshooting | 591 |
| 24.3.12.1 | Phage Titers are not Reproducible | 591 |
| 24.3.12.2 | Displayed Protein is Degrading with Time | 592 |
| 24.3.12.3 | Phages are not Genetically Stable | 592 |
| 24.3.12.4 | The Ratio 'Out/In' is not Increasing with the Selection Rounds | 592 |
| 24.4 | Conclusions and Future Challenges | 592 |
| | References | 593 |
| 25 | Screening Methodologies for Glycosidic Bond Formation | 605 |
| | <i>Amir Aharoni and Stephen G. Withers</i> | |
| 25.1 | Introduction | 605 |

| | | |
|---------|---|-----|
| 25.2 | Glycosynthases | 607 |
| 25.3 | Glycosyltransferases | 608 |
| 25.4 | Protocol and Practical Considerations for Using HTS Methodology in the Directed Evolution of STs | 610 |
| 25.4.1 | Cloning of the Target ST and CMP-Neu5Ac-Synthetase | 610 |
| 25.4.2 | Synthesis of Fluorescently Labeled Acceptor Sugar | 611 |
| 25.4.3 | Cell-Based Assay in JM107 <i>Nan</i> A ⁻ Strain | 611 |
| 25.4.4 | Transformation, Growth and Expression of Plasmids Containing ST and CMP-syn Genes in JM107 <i>Nan</i> A ⁻ Strain | 612 |
| 25.4.5 | Cell-Based Assay | 613 |
| 25.4.6 | Validation, Sensitivity and Dynamic Range of the Cell-Based Assay | 613 |
| 25.4.7 | Model Selection | 614 |
| 25.4.8 | Generation of Genetic Diversity in the Target ST Gene: Strategies for Constructing Large Mutant Libraries | 614 |
| 25.4.9 | Library Sorting, Rounds of Enrichment and the Stringency of Selection | 615 |
| 25.4.10 | Identification and Isolation of Improved Mutants | 615 |
| 25.4.11 | Characterization of Improved ST Mutants | 616 |
| 25.5 | Challenges and Prospects of GT Engineering | 617 |
| | References | 617 |

26 Yeast Surface Display in Protein Engineering and Analysis 621

Benjamin J. Hackel and K. Dane Wittrup

| | | |
|----------|--|-----|
| 26.1 | Review | 621 |
| 26.1.1 | Introduction | 621 |
| 26.1.2 | Protein Engineering | 622 |
| 26.1.2.1 | Affinity Engineering | 623 |
| 26.1.2.2 | Stability and Expression Engineering | 623 |
| 26.1.2.3 | Enzyme Engineering | 624 |
| 26.1.3 | Protein Analysis | 624 |
| 26.1.3.1 | Clone Characterization | 624 |
| 26.1.3.2 | Paratope: Epitope Study | 625 |
| 26.1.3.3 | YSD in Bioassays | 626 |
| 26.2 | Protocols and Practical Considerations | 626 |
| 26.2.1 | Materials | 627 |
| 26.2.1.1 | Cells and Plasmids | 627 |
| 26.2.1.2 | Media and Buffers | 627 |
| 26.2.1.3 | Buffers | 627 |
| 26.2.1.4 | Flow Cytometry Reagents | 627 |
| 26.2.2 | Nucleic Acid and Yeast Preparation | 628 |
| 26.2.2.1 | DNA Preparation | 628 |
| 26.2.2.2 | Yeast Transformation | 630 |
| 26.2.2.3 | Yeast Culture | 632 |
| 26.2.3 | Combinatorial Library Selection | 632 |

| | | |
|-----------|--|------------|
| 26.2.4 | FACS | 633 |
| 26.2.4.1 | Other Selection Techniques | 635 |
| 26.2.4.2 | Stability | 636 |
| 26.2.4.3 | Clone Identification | 637 |
| 26.2.5 | Analysis | 637 |
| 26.2.5.1 | Binding Measurements | 637 |
| 26.2.5.2 | Stability Measurement | 641 |
| 26.3 | The Future of Yeast Surface Display | 642 |
| | Abbreviations | 644 |
| | Acknowledgments | 644 |
| | References | 644 |
| 27 | In Vitro Compartmentalization (IVC) and Other High-Throughput Screens of Enzyme Libraries | 649 |
| | <i>Amir Aharoni and Dan S. Tawfik</i> | |
| 27.1 | Introduction | 649 |
| 27.2 | The Fundamentals of High-Throughput Screens and Selections | 650 |
| 27.3 | Enzyme Selections by Phage-Display | 651 |
| 27.4 | HTS of Enzymes Using Cell-Display and FACS | 652 |
| 27.5 | Other FACS-Based Enzyme Screens | 653 |
| 27.6 | <i>In vivo</i> Genetic Screens and Selections | 653 |
| 27.7 | <i>In vitro</i> Compartmentalization (IVC) | 654 |
| 27.8 | IVC in Double Emulsions | 657 |
| 27.9 | What's Next? | 659 |
| 27.10 | Experimental Details | 660 |
| | Acknowledgments | 662 |
| | References | 662 |
| 28 | Colorimetric and Fluorescence-Based Screening | 669 |
| | <i>Jean-Louis Reymond</i> | |
| 28.1 | Introduction | 669 |
| 28.2 | Enzyme-Coupled Assays | 670 |
| 28.2.1 | Alcohol Dehydrogenase (ADH)-Coupled Assays | 671 |
| 28.2.2 | Peroxidase-Coupled Assays | 673 |
| 28.2.3 | Hydrolase-Coupled Assays | 674 |
| 28.2.4 | Luciferase-Coupled Assays | 676 |
| 28.3 | Fluorogenic and Chromogenic Substrates | 678 |
| 28.3.1 | Release of Aromatic Alcohols | 678 |
| 28.3.2 | Aniline Release | 681 |
| 28.3.3 | FRET | 682 |
| 28.3.4 | Reactions that Modify the Chromophore Directly | 685 |
| 28.3.5 | Separation of Labeled Substrates | 685 |
| 28.3.6 | Precipitation | 687 |
| 28.4 | Chemosensors and Biosensors | 688 |
| 28.4.1 | Quick-E with pH-Indicators | 688 |

| | | |
|-----------|--|------------|
| 28.4.2 | Functional Group-Selective Reagents | 689 |
| 28.4.3 | Antibodies, Aptamers and Lectins | 690 |
| 28.4.4 | Gold Nanoparticles | 691 |
| 28.5 | Enzyme Fingerprinting with Multiple Substrates | 693 |
| 28.5.1 | APIZYM | 693 |
| 28.5.2 | Protease Profiling | 695 |
| 28.5.3 | Cocktail Fingerprinting | 695 |
| 28.5.4 | Substrate Microarrays | 697 |
| 28.6 | Conclusions | 698 |
| | Acknowledgments | 699 |
| | References | 699 |
| 29 | Confocal and Conventional Fluorescence-Based High Throughput Screening in Protein Engineering | 713 |
| | <i>Ulrich Haupts, Oliver Hesse, Michael Strerath, Peter J. Walla, and Wayne M. Coco</i> | |
| 29.1 | General Aspects | 713 |
| 29.1.1 | HTS and Combinatorial DNA Library Strategies in Protein Engineering | 713 |
| 29.1.2 | HTS in Protein Engineering: Coupling Genotype and Phenotype and the Advantages of Clonal Assays | 715 |
| 29.1.3 | Well-Based HTS Formats | 716 |
| 29.2 | Fluorescence | 718 |
| 29.2.1 | Overview of Theory and Principles of Fluorescence | 719 |
| 29.2.1.1 | Choice of Fluorophores in HTS | 721 |
| 29.2.1.2 | Concentration Requirements for Fluorescent Analytes | 722 |
| 29.2.1.3 | Fluorescence Intensity Measurements with a Precautionary Note on Fluorescent Labeling of Substrates and Binding Partners | 722 |
| 29.2.1.4 | Confocal Versus Bulk Detection Methods | 723 |
| 29.2.1.5 | Advantages of the Confocal Fluorescence Detection Format | 724 |
| 29.2.1.6 | Anisotropy | 724 |
| 29.2.1.7 | FRET/TR-FRET/Lifetime | 725 |
| 29.2.1.8 | Fluorescence Correlation Spectroscopy | 726 |
| 29.2.1.9 | FIDA | 726 |
| 29.3 | Hardware and Instrumentation | 727 |
| 29.3.1 | Confocal and Bulk Concepts | 727 |
| 29.3.1.1 | Light Sources | 727 |
| 29.3.1.2 | Wavelength Selection/Filtering | 729 |
| 29.3.1.3 | Detectors | 729 |
| 29.3.1.4 | Reader Systems | 730 |
| 29.4 | Practical Considerations and Screening Protocol | 730 |
| 29.4.1 | Introduction | 730 |
| 29.4.2 | Fluorescence-Based Assay Design: Practical Considerations | 731 |
| 29.4.2.1 | Choice of Assay Design | 731 |
| 29.4.2.2 | Labeling | 731 |

| | | |
|----------|--|-----|
| 29.4.2.3 | Choice of Fluorophore | 732 |
| 29.4.3 | Assay Quality | 733 |
| 29.4.3.1 | What Needs to Be Discriminated? | 733 |
| 29.4.3.2 | Mathematical Description | 733 |
| 29.4.4 | A Specific HTS Protein Engineering Program Using a Fluorescence-Based Screen | 735 |
| 29.4.5 | The Assay | 735 |
| 29.4.5.1 | Expression Host | 736 |
| 29.4.6 | Multiwell Format and Unit Operations in the HTS Protocol | 738 |
| 29.4.6.1 | Liquid Handling | 738 |
| 29.4.6.2 | Incubation | 738 |
| 29.4.6.3 | Centrifugation | 739 |
| 29.4.6.4 | Scheduling | 739 |
| 29.4.6.5 | Screening Protocol | 739 |
| 29.5 | Challenges and Future Directions | 742 |
| | Abbreviations | 748 |
| | Acknowledgments | 748 |
| | References | 748 |

30 Alteration of Substrate Specificity and Stereoselectivity of Lipases and Esterases 753

Dominique Böttcher, Marlen Schmidt, and Uwe T. Bornscheuer

| | | |
|----------|---|-----|
| 30.1 | Introduction | 753 |
| 30.2 | Background of Protein Engineering Methods | 754 |
| 30.2.1 | Directed Evolution | 754 |
| 30.2.2 | Rational Design | 756 |
| 30.3 | Assay Systems | 757 |
| 30.3.1 | Selection | 757 |
| 30.3.1.1 | Display Techniques | 757 |
| 30.3.1.2 | <i>In vivo</i> Selection | 758 |
| 30.3.2 | Screening | 759 |
| 30.4 | Examples | 764 |
| 30.5 | Conclusions | 770 |
| | References | 770 |

31 Altering Enzyme Substrate and Cofactor Specificity via Protein Engineering 777

Matthew DeSieno, Jing Du, and Huimin Zhao

| | | |
|----------|----------------------|-----|
| 31.1 | Introduction | 777 |
| 31.1.1 | Overview | 777 |
| 31.1.2 | Approaches | 779 |
| 31.1.2.1 | Rational Design | 779 |
| 31.1.2.2 | Directed Evolution | 781 |
| 31.1.2.3 | Semi-Rational Design | 781 |
| 31.2 | Specific Examples | 782 |

| | | |
|-----------|---|------------|
| 31.2.1 | Cofactor Specificity | 782 |
| 31.2.1.1 | NAD(P)(H) | 783 |
| 31.2.1.2 | ATP | 783 |
| 31.2.1.3 | Summary and Comments for Cofactor Specificity | 784 |
| 31.2.2 | Substrate Specificity | 784 |
| 31.2.2.1 | P450s | 785 |
| 31.2.2.2 | Aldolases | 785 |
| 31.2.2.3 | Transfer-RNA Synthetases | 786 |
| 31.2.2.4 | Restriction Endonucleases | 786 |
| 31.2.2.5 | Homing Endonucleases | 788 |
| 31.2.2.6 | Polymerases | 789 |
| 31.2.2.7 | Summary and Comments for Substrate Specificity | 789 |
| 31.3 | Challenges and Future Prospects | 790 |
| 31.3.1 | New Strategies for Engineering Cofactor/Substrate Specificity | 790 |
| 31.3.2 | Cofactor/Substrate Specificity Engineering for Combinatorial Biosynthesis | 791 |
| 31.3.3 | Cofactor/Substrate Specificity Engineering for Metabolic Engineering | 792 |
| 31.3.4 | Cofactor/Substrate Specificity Engineering for Gene Therapy | 793 |
| | Acknowledgments | 793 |
| | References | 793 |
| 32 | Protein Engineering of Modular Polyketide Synthases | 797 |
| | <i>Alice Y. Chen and Chaitan Khosla</i> | |
| 32.1 | Introduction | 797 |
| 32.2 | Polyketide Biosynthesis and Engineering | 798 |
| 32.2.1 | Active Sites and Domain Boundaries in Multimodular PKSs | 799 |
| 32.2.2 | Past Achievements in Genetic Reprogramming of Polyketide Biosynthesis | 802 |
| 32.2.2.1 | Starter Unit Incorporation | 802 |
| 32.2.2.2 | Extender Unit Incorporation | 804 |
| 32.2.2.3 | β -Carbon Processing | 805 |
| 32.2.2.4 | Chain Length Control | 807 |
| 32.2.2.5 | Additional Modifications | 807 |
| 32.2.2.6 | Other PKS Engineering Opportunities | 807 |
| 32.2.3 | Pre-/Post-PKS Pathway Engineering | 809 |
| 32.2.3.1 | Precursor Production | 809 |
| 32.2.3.2 | Post-PKS Modification | 810 |
| 32.3 | Engineering and Characterization Techniques | 810 |
| 32.3.1 | Common Genetic Techniques for PKS Engineering | 810 |
| 32.3.1.1 | Restriction Site Engineering | 811 |
| 32.3.1.2 | Gene SOEing | 811 |
| 32.3.1.3 | Red/ET Homology Recombination | 811 |
| 32.3.1.4 | Gene Synthesis | 812 |
| 32.3.1.5 | Gene Shuffling | 813 |

| | | |
|-----------|--|-----|
| 32.3.2 | <i>In vitro</i> Characterization | 814 |
| 32.3.2.1 | Protein Expression | 814 |
| 32.3.2.2 | Protein Purification | 814 |
| 32.3.2.3 | Protein Characterization | 815 |
| 32.3.3 | <i>In vivo</i> Characterization | 816 |
| 32.3.3.1 | Host Engineering | 816 |
| 32.3.3.2 | High-Throughput Screening Assay | 817 |
| 32.4 | The Path Forward | 818 |
| | Abbreviations | 819 |
| | References | 819 |
| 33 | Cyanophycin Synthetases | 829 |
| | <i>Anna Steinle and Alexander Steinbüchel</i> | |
| 33.1 | Introduction | 829 |
| 33.2 | Occurrence of Cyanophycin Synthetases | 830 |
| 33.3 | General Features | 830 |
| 33.4 | Reaction Mechanism | 831 |
| 33.5 | Substrate Specificity | 832 |
| 33.6 | Primary Structure Analysis | 836 |
| 33.7 | Enzyme Engineering | 838 |
| 33.8 | Biotechnical Applications | 843 |
| | Acknowledgments | 843 |
| | References | 843 |
| 34 | Biosynthetic Pathway Engineering Strategies | 849 |
| | <i>Claudia Schmidt-Dannert and Alexander Pisarchik</i> | |
| 34.1 | Introduction | 849 |
| 34.2 | Initial Pathway Design | 850 |
| 34.2.1 | Functional Pathway Assembly | 850 |
| 34.2.2 | Selection of the Heterologous Host | 854 |
| 34.3 | Optimization of the Precursor Supply | 855 |
| 34.3.1 | Identification and Overexpression of Rate-Limiting Enzymes | 856 |
| 34.4 | Engineering of Control Loops | 858 |
| 34.5 | Engineering of Alternative Precursor Routes | 858 |
| 34.6 | Balancing Gene Expression Levels and Activities of Metabolic Enzymes | 859 |
| 34.7 | Metabolic Network Integration and Optimization | 861 |
| 34.8 | Engineering Pathways for the Production of Diverse Compounds | 863 |
| 34.9 | Future Perspectives | 866 |
| | Abbreviations | 867 |
| | References | 868 |

| | |
|---------------|--|
| 35 | Natural Polyester-Related Proteins: Structure, Function, Evolution and Engineering 877 |
| | <i>Seiichi Taguchi and Takeharu Tsuge</i> |
| 35.1 | Introduction 877 |
| 35.2 | Enzymes Related to the Synthesis and Degradation of PHA 878 |
| 35.3 | Structure-Based Engineering of PHA Synthase and Monomer-Supplying Enzymes 879 |
| 35.3.1 | PHA Synthase (PhaC, PhaEC, PhaRC) 880 |
| 35.3.2 | 3-Ketoacyl-CoA Thiolase (PhaA) 882 |
| 35.3.3 | Acetoacetyl-CoA Reductase (PhaB) 887 |
| 35.3.4 | (<i>R</i>)-Specific Enoyl-CoA Hydratase (PhaJ) 890 |
| 35.3.5 | (<i>R</i>)-3-Hydroxyacyl-ACP-CoA Transferase (PhaG) 891 |
| 35.3.6 | 3-Ketoacyl-ACP Synthase III (FabH) 891 |
| 35.4 | Directed Evolution of PHA Synthases 892 |
| 35.4.1 | Engineering of the Type I Synthases 893 |
| 35.4.2 | Engineering of the Type II <i>Pseudomonas</i> Species PHA Synthases 897 |
| 35.5 | Structure–Function Relationship of PHA Depolymerases 899 |
| 35.5.1 | Domain Structure of Extracellular PHA Depolymerases 899 |
| 35.5.2 | Intracellular PHA Depolymerase 903 |
| 35.5.3 | Amino Acid Residues Related to Binding Affinity 904 |
| 35.6 | Application of PHA-Protein Binding Affinity 905 |
| 35.7 | Perspectives 906 |
| | References 907 |
| 36 | Bioengineering of Sequence-Repetitive Polypeptides: Synthetic Routes to Protein-Based Materials of Novel Structure and Function 915 |
| | <i>Sonha C. Payne, Melissa Patterson, and Vincent P. Conticello</i> |
| 36.1 | Introduction 915 |
| 36.2 | Block Copolymers as Targets for Materials Design 918 |
| 36.2.1 | Amphiphilic Block Copolymers 919 |
| 36.2.2 | Elastin-Mimetic Block Copolymers 920 |
| 36.3 | Strategies for the Construction of Synthetic Genes Encoding Sequence-Repetitive Polypeptides 923 |
| 36.3.1 | DNA Cassette Concatemerization 924 |
| 36.3.2 | Recursive Directional Ligation 925 |
| 36.3.3 | Genetic Assembly of Synthetic Genes Encoding Block Architectures 926 |
| 36.4 | A Hybrid Approach to the Controlled Assembly of Complex Architectures of Sequence-Repetitive Polypeptides 928 |
| 36.5 | Future Outlook 935 |
| | Acknowledgments 936 |
| | References 936 |

| | | |
|-----------|--|------------|
| 37 | Silk Proteins—Biomaterials and Bioengineering | 939 |
| | <i>Xiaoqin Wang, Peggy Cebe, and David. L. Kaplan</i> | |
| 37.1 | Silk Protein Polymers—An Overview | 939 |
| 37.2 | Silk Protein Polymers—Methods of Preparation | 947 |
| 37.2.1 | Preparation of Spider Silks | 947 |
| 37.2.2 | Preparation of Scaffolds | 949 |
| 37.3 | Silk Protein Polymers—Future Perspectives and Challenges | 951 |
| | Acknowledgments | 954 |
| | References | 954 |
| | Index | 961 |

