Contents

	Foreword xv	2.5.1	DNA Nanotechnology 18
	Preface xvii	2.5.2	DNA-Templated Nanoparticle Assembly 19
	About the Companion Website xix	2.5.3	DNA Nanomachines 20
	·	2.5.4	DNA Nanotechnology for Biology 20
1	Introduction to Advanced Chemical	2.5.5	DNA-Based Organelle Mapping
	Biology 1		Technology 20
	Howard C. Hang, Matthew R. Pratt, and	2.5.6	DNA-Based Technologies for the Detection of
	Jennifer A. Prescher		Endogenous Nucleic Acids and Proteins 22
1.1	Introduction 1	2.5.6.1	Fluorescence In Situ Hybridization
1.2	Enabled by Synthetic and Physical Organic		(FISH) 22
	Chemistry 1	2.5.6.2	DNA-Barcoded Antibodies for Spatial
1.3	Guided by Biochemistry and Structural		Detection of Proteins 22
	Biology 3	2.5.7	DNA-Based Super Resolution Imaging 22
1.4	Enhanced by Engineering and Evolution 3	2.5.8	DNA-Encoded Libraries (DEL) 23
1.5	Expanded by Analytical Chemistry and	2.5.9	Digital Data Storage Using DNA 23
	"Omics" Technologies 4	2.6	Tools for Engineering DNA 24
1.6	Impact on Biological Discovery and Drug	2.7	Summary and Future Outlook 25
	Development 5		Acknowledgments 25
1.7	Outlook 5		Questions 25
	References 6		References 26
2	DNA Function, Synthesis, and		
	Engineering 9	3	Chemical Approaches to Genome
	Engineering 9 Aneesh T. Veetil and Yamuna Krishnan	3	Chemical Approaches to Genome Integrity 31
2.1	•	3	• •
2.1 2.1.1	Aneesh T. Veetil and Yamuna Krishnan	3	Integrity 31
	Aneesh T. Veetil and Yamuna Krishnan Introduction: A Historical Perspective 9	3 .1	Integrity 31 Elizabeth R. Lotsof, Savannah G. Conlon, and Sheila S. David Introduction and Historical Perspective 31
2.1.1	Aneesh T. Veetil and Yamuna Krishnan Introduction: A Historical Perspective 9 The Structure of DNA 9		Integrity 31 Elizabeth R. Lotsof, Savannah G. Conlon, and Sheila S. David Introduction and Historical Perspective 31 Types of DNA Damage 32
2.1.1	Aneesh T. Veetil and Yamuna Krishnan Introduction: A Historical Perspective 9 The Structure of DNA 9 New Nucleobases and Unusual DNA	3.1	Integrity 31 Elizabeth R. Lotsof, Savannah G. Conlon, and Sheila S. David Introduction and Historical Perspective 31
2.1.1 2.2	Aneesh T. Veetil and Yamuna Krishnan Introduction: A Historical Perspective 9 The Structure of DNA 9 New Nucleobases and Unusual DNA Conformations 11	3.1 3.2	Integrity 31 Elizabeth R. Lotsof, Savannah G. Conlon, and Sheila S. David Introduction and Historical Perspective 31 Types of DNA Damage 32 Damage to Nucleobase 32 Oxidation 32
2.1.1 2.2 2.2.1	Aneesh T. Veetil and Yamuna Krishnan Introduction: A Historical Perspective 9 The Structure of DNA 9 New Nucleobases and Unusual DNA Conformations 11 G-Quadruplex DNA Structures 11	3.1 3.2 3.2.1	Integrity 31 Elizabeth R. Lotsof, Savannah G. Conlon, and Sheila S. David Introduction and Historical Perspective 31 Types of DNA Damage 32 Damage to Nucleobase 32 Oxidation 32 Alkylation 33
2.1.1 2.2 2.2.1 2.2.2	Aneesh T. Veetil and Yamuna Krishnan Introduction: A Historical Perspective 9 The Structure of DNA 9 New Nucleobases and Unusual DNA Conformations 11 G-Quadruplex DNA Structures 11 Circular DNA Structures 11	3.1 3.2 3.2.1 3.2.1.1	Integrity 31 Elizabeth R. Lotsof, Savannah G. Conlon, and Sheila S. David Introduction and Historical Perspective 31 Types of DNA Damage 32 Damage to Nucleobase 32 Oxidation 32
2.1.1 2.2 2.2.1 2.2.2 2.2.3	Aneesh T. Veetil and Yamuna Krishnan Introduction: A Historical Perspective 9 The Structure of DNA 9 New Nucleobases and Unusual DNA Conformations 11 G-Quadruplex DNA Structures 11 Circular DNA Structures 11 Aptamers 11	3.1 3.2 3.2.1 3.2.1.1 3.2.1.2	Integrity 31 Elizabeth R. Lotsof, Savannah G. Conlon, and Sheila S. David Introduction and Historical Perspective 31 Types of DNA Damage 32 Damage to Nucleobase 32 Oxidation 32 Alkylation 33
2.1.1 2.2 2.2.1 2.2.2 2.2.3 2.2.4	Aneesh T. Veetil and Yamuna Krishnan Introduction: A Historical Perspective 9 The Structure of DNA 9 New Nucleobases and Unusual DNA Conformations 11 G-Quadruplex DNA Structures 11 Circular DNA Structures 11 Aptamers 11 Other Nucleobases 12	3.1 3.2 3.2.1 3.2.1.1 3.2.1.2 3.2.1.3	Integrity 31 Elizabeth R. Lotsof, Savannah G. Conlon, and Sheila S. David Introduction and Historical Perspective 31 Types of DNA Damage 32 Damage to Nucleobase 32 Oxidation 32 Alkylation 33 Depurination/Depyrimidination 35
2.1.1 2.2 2.2.1 2.2.2 2.2.3 2.2.4 2.3	Aneesh T. Veetil and Yamuna Krishnan Introduction: A Historical Perspective 9 The Structure of DNA 9 New Nucleobases and Unusual DNA Conformations 11 G-Quadruplex DNA Structures 11 Circular DNA Structures 11 Aptamers 11 Other Nucleobases 12 The Modern Synthesis of DNA 13 Solid-Phase DNA Synthesis 13 Backbone-Modified Oligonucleotides 15	3.1 3.2 3.2.1.1 3.2.1.2 3.2.1.3 3.2.1.4 3.2.1.5 3.2.1.6	Integrity 31 Elizabeth R. Lotsof, Savannah G. Conlon, and Sheila S. David Introduction and Historical Perspective 31 Types of DNA Damage 32 Damage to Nucleobase 32 Oxidation 32 Alkylation 33 Depurination/Depyrimidination 35 Deamination 35 DNA Mismatches 35 DNA Crosslinks 35
2.1.1 2.2 2.2.1 2.2.2 2.2.3 2.2.4 2.3 2.3.1	Aneesh T. Veetil and Yamuna Krishnan Introduction: A Historical Perspective 9 The Structure of DNA 9 New Nucleobases and Unusual DNA Conformations 11 G-Quadruplex DNA Structures 11 Circular DNA Structures 11 Aptamers 11 Other Nucleobases 12 The Modern Synthesis of DNA 13 Solid-Phase DNA Synthesis 13	3.1 3.2 3.2.1 3.2.1.1 3.2.1.2 3.2.1.3 3.2.1.4 3.2.1.5	Integrity 31 Elizabeth R. Lotsof, Savannah G. Conlon, and Sheila S. David Introduction and Historical Perspective 31 Types of DNA Damage 32 Damage to Nucleobase 32 Oxidation 32 Alkylation 33 Depurination/Depyrimidination 35 Deamination 35 DNA Mismatches 35 DNA Crosslinks 35 Damage to Sugar 36
2.1.1 2.2 2.2.1 2.2.2 2.2.3 2.2.4 2.3 2.3.1 2.3.2	Aneesh T. Veetil and Yamuna Krishnan Introduction: A Historical Perspective 9 The Structure of DNA 9 New Nucleobases and Unusual DNA Conformations 11 G-Quadruplex DNA Structures 11 Circular DNA Structures 11 Aptamers 11 Other Nucleobases 12 The Modern Synthesis of DNA 13 Solid-Phase DNA Synthesis 13 Backbone-Modified Oligonucleotides 15 Peptide Nucleic Acids (PNAs) 16 Morpholino Nucleic Acids 16	3.1 3.2 3.2.1.1 3.2.1.2 3.2.1.3 3.2.1.4 3.2.1.5 3.2.1.6	Integrity 31 Elizabeth R. Lotsof, Savannah G. Conlon, and Sheila S. David Introduction and Historical Perspective 31 Types of DNA Damage 32 Damage to Nucleobase 32 Oxidation 32 Alkylation 33 Depurination/Depyrimidination 35 Deamination 35 DNA Mismatches 35 DNA Crosslinks 35 Damage to Sugar 36 Damage to Phosphate Backbone 36
2.1.1 2.2 2.2.1 2.2.2 2.2.3 2.2.4 2.3 2.3.1 2.3.2 2.3.2,1	Aneesh T. Veetil and Yamuna Krishnan Introduction: A Historical Perspective 9 The Structure of DNA 9 New Nucleobases and Unusual DNA Conformations 11 G-Quadruplex DNA Structures 11 Circular DNA Structures 11 Aptamers 11 Other Nucleobases 12 The Modern Synthesis of DNA 13 Solid-Phase DNA Synthesis 13 Backbone-Modified Oligonucleotides 15 Peptide Nucleic Acids (PNAs) 16 Morpholino Nucleic Acids 16 DNA Sequencing 16	3.1 3.2 3.2.1.1 3.2.1.2 3.2.1.3 3.2.1.4 3.2.1.5 3.2.1.6 3.2.2	Integrity 31 Elizabeth R. Lotsof, Savannah G. Conlon, and Sheila S. David Introduction and Historical Perspective 31 Types of DNA Damage 32 Damage to Nucleobase 32 Oxidation 32 Alkylation 33 Depurination/Depyrimidination 35 Deamination 35 DNA Mismatches 35 DNA Crosslinks 35 Damage to Sugar 36 Damage to Phosphate Backbone 36 Types of DNA Repair 36
2.1.1 2.2 2.2.1 2.2.2 2.2.3 2.2.4 2.3 2.3.1 2.3.2 2.3.2.1 2.3.2.2	Aneesh T. Veetil and Yamuna Krishnan Introduction: A Historical Perspective 9 The Structure of DNA 9 New Nucleobases and Unusual DNA Conformations 11 G-Quadruplex DNA Structures 11 Circular DNA Structures 11 Aptamers 11 Other Nucleobases 12 The Modern Synthesis of DNA 13 Solid-Phase DNA Synthesis 13 Backbone-Modified Oligonucleotides 15 Peptide Nucleic Acids (PNAs) 16 Morpholino Nucleic Acids 16 DNA Sequencing 16 Modern Methods to Sequence DNA 16	3.1 3.2 3.2.1.1 3.2.1.2 3.2.1.3 3.2.1.4 3.2.1.5 3.2.1.6 3.2.2 3.2.3 3.3 3.3.1	Integrity 31 Elizabeth R. Lotsof, Savannah G. Conlon, and Sheila S. David Introduction and Historical Perspective 31 Types of DNA Damage 32 Damage to Nucleobase 32 Oxidation 32 Alkylation 33 Depurination/Depyrimidination 35 Deamination 35 DNA Mismatches 35 DNA Crosslinks 35 Damage to Sugar 36 Damage to Phosphate Backbone 36 Types of DNA Repair 36 Direct Repair 36
2.1.1 2.2 2.2.1 2.2.2 2.2.3 2.2.4 2.3 2.3.1 2.3.2 2.3.2.1 2.3.2.2 2.4	Aneesh T. Veetil and Yamuna Krishnan Introduction: A Historical Perspective 9 The Structure of DNA 9 New Nucleobases and Unusual DNA Conformations 11 G-Quadruplex DNA Structures 11 Circular DNA Structures 11 Aptamers 11 Other Nucleobases 12 The Modern Synthesis of DNA 13 Solid-Phase DNA Synthesis 13 Backbone-Modified Oligonucleotides 15 Peptide Nucleic Acids (PNAs) 16 Morpholino Nucleic Acids 16 DNA Sequencing 16 Modern Methods to Sequence DNA 16 Sequencing by Synthesis (SBS) 17	3.1 3.2 3.2.1.1 3.2.1.2 3.2.1.3 3.2.1.4 3.2.1.5 3.2.1.6 3.2.2 3.2.3 3.3	Integrity 31 Elizabeth R. Lotsof, Savannah G. Conlon, and Sheila S. David Introduction and Historical Perspective 31 Types of DNA Damage 32 Damage to Nucleobase 32 Oxidation 32 Alkylation 33 Depurination/Depyrimidination 35 Deamination 35 DNA Mismatches 35 DNA Crosslinks 35 Damage to Sugar 36 Damage to Phosphate Backbone 36 Types of DNA Repair 36 Direct Repair 36 Base Excision Repair 38
2.1.1 2.2 2.2.1 2.2.2 2.2.3 2.2.4 2.3 2.3.1 2.3.2 2.3.2.1 2.3.2.2 2.4 2.4.1	Aneesh T. Veetil and Yamuna Krishnan Introduction: A Historical Perspective 9 The Structure of DNA 9 New Nucleobases and Unusual DNA Conformations 11 G-Quadruplex DNA Structures 11 Circular DNA Structures 11 Aptamers 11 Other Nucleobases 12 The Modern Synthesis of DNA 13 Solid-Phase DNA Synthesis 13 Backbone-Modified Oligonucleotides 15 Peptide Nucleic Acids (PNAs) 16 Morpholino Nucleic Acids 16 DNA Sequencing 16 Modern Methods to Sequence DNA 16	3.1 3.2 3.2.1.1 3.2.1.2 3.2.1.3 3.2.1.4 3.2.1.5 3.2.1.6 3.2.2 3.2.3 3.3 3.3.1	Integrity 31 Elizabeth R. Lotsof, Savannah G. Conlon, and Sheila S. David Introduction and Historical Perspective 31 Types of DNA Damage 32 Damage to Nucleobase 32 Oxidation 32 Alkylation 33 Depurination/Depyrimidination 35 Deamination 35 DNA Mismatches 35 DNA Crosslinks 35 Damage to Sugar 36 Damage to Phosphate Backbone 36 Types of DNA Repair 36 Direct Repair 36

3.3.5	Double-Strand Break and Interstrand Crosslink Repair 39	4.5	Identification and Engineering of Functional RNAs 62
3.4	Identification of Sites of DNA Damage and	4.5.1	Aptamers 62
	Modification 40	4.5.2	Riboswitches 63
3.4.1	Traditional Methods for Damage	4.5.3	Ribozymes 63
	Detection 40	4.5.4	Genetically Encoded Tags to Label RNA 64
3.4.2	Searching for Hotspots of Oxidative	4.5.5	RNA-Based Therapeutics 64
	Damage – An OG Story 41	4.6	The Sequencing of RNA 64
3.4.3	Sequencing for Bulky Adducts – Cisplatin and	4.6.1	Reverse Transcription of RNA 65
	Pyrimidine Dimers 41	4.6.2	Long-Read and Direct RNA Sequencing 65
3.4.4	Sequencing for AP Site and Strand Breaks 44	4.6.3	Extensions and Alternative Approaches to
3.5	Assays that Allow for Monitoring of the		RNA-seq 66
	Repair of DNA Damage in Cellular	4.7	The Chemical Probing of RNA Structure 66
	Contexts 44	4.7.1	In-Line Probing of RNA Conformation 67
3.5.1	Lesion Reporter Assays to Monitor Base	4.7.2	Reagents for Chemical Probing of RNA
	Excision Repair 45		Conformation and Base-Pairing 67
3.5.2	Leveraging Cell-Based Reporter Assays to	4.7.3	Reagents for Probing Solvent Accessibility,
	Assess Impact of DNA Lesions on Replication	,.5	Tertiary Structure, and Higher Order
	and Transcription 47		Interactions 68
3.5.3	Plasmid Reporters Monitoring Several DNA	4.8	Summary and Future Outlook 69
	Repair Pathways Simultaneously 47		Questions 69
3.5.4	Highly Sensitive Fluorescent DNA Repair		References 69
	Probes for Clinical Diagnostics and Imaging		References
	in Cells 48	5	Chemical Approaches to
3.6	Summary and Future Outlook 48	•	Transcription and RNA Regulation
2.0	Acknowledgments 48		In Vivo 75
	Exam Questions 48		Tong Wu and Chuan He
	References 50	5.1	Introduction/Historical Perspective 75
		5.2	Core Concepts/Landmark Studies 75
4	RNA Function, Synthesis, and	5.2.1	Transcription Regulation in Eukaryotes 75
•	Probing 55	5.3	Transcription Regulation by Chemical
	Andreas Pintado-Urbanc and Matthew D. Simon		Targeting of DNA and the Core Transcription
4.1	Introduction 55		Machinery 76
4.2	The Principles of RNA Chemistry 56	5.3.1	Cell-Permeable DNA-Targeting Small
4.2.1	The Impact of a 2'-Hydroxyl on Nucleic Acid	5.5.1	Molecules 76
	Chemistry 56	5.3.2	Targeting Transcription by Nucleic Acids and
4.2.2	RNA Bases and Base-Pairing 56		Their Analogs 79
4.2.3	RNA Secondary Structure 58	5.3.3	Small-Molecule Inhibitors of the
4.2.4	RNA Tertiary Structures and the		Transcription Machinery 80
	Ribosome 58	5.4	Chemical Regulation of Transcription via
4.3	Synthesis of RNA 58		Targeting of Epigenetic Elements 81
4.3.1	Chemical Synthesis 58	5.4.1	Transcription Regulation Through Targeting
4.3.2	In Vitro Transcription 59		of Histone Modifications 81
4.4	Labeling of RNA 60	5.4.2	DNA Methylation and Small Molecules
4.4.1	Introducing Modifications Through Chemical		Targeting DNA Modifications 83
	Synthesis of RNA 60	5.5	Chemical Approaches to Target
4.4.2	Using Ligation to Introduce Chemical		Post-Transcriptional RNA Metabolism 86
	Modifications into RNA 60	5.5.1	Post-Transcriptional RNA Metabolism 86
4.4.3	Incorporation of Modified Bases into RNA	5.5.2	Regulating RNA Function by Direct RNA
	Using IVT 61		Binders 89
4.4.4	Approaches to 3'-End Label RNA 62	5.5.3	Regulating RNA Function by Targeting
4.4.5	Approaches to 5'-End Label RNA 62		RNA-Binding (Effector) Proteins 91

5.6	Summary and Future Outlook 91	7.2.1	SPPS Is Optimized for Stepwise
	Questions 92		Efficiency 135
	References 92	7.2.2	N^{α} -protecting Groups Ensure Single
			Coupling of the Incoming Amino Acid 136
6	Chemical Biology of Genome	7.2.3	Plastic Resins Are Used During SPPS 138
0	Engineering 99	7.2.4	Temporary Masking of Reactive Side Chains
	Carlos A. Vasquez and Alexis C. Komor		Is Necessary During SPPS 138
6.1	Introduction to Genome Editing 99	7.2.5	Peptide Bonds Are Synthesized by a
6.2	Early Genetic Engineering Experiments:		Condensation Reaction Mediated by a
0.2	Chemical Mutagenesis, Gene Transfer, and		Stoichiometric Coupling Agent 140
	Gene Targeting 100	7.3	Secondary and Tertiary Structures of Amino
6.2.1	Chemical Mutagenesis Methods 100		Acids 142
6.2.2	Gene Transfer 101	7.3.1	Peptide Backbone Conformations 142
6.2.3		7.3.2	Biophysical Determinants of Helix Folding
	Gene Targeting 102 Improving Precision and Programmability		and Design of α-Helix Mimics 143
6.3	with Double-Stranded DNA Breaks 103	7.3.3	β-Strand and β-Sheet Mimics 145
621		7.3.4	Protein Tertiary Structure Mimics 147
6.3.1	The Development of Double-Stranded	7.3.4.1	β-Sheet and β-Hairpin Mimics 147
	Break-Reliant Genome Editing	7.3.5	Helical Tertiary Structure Mimics 149
(22	Technologies 103	7.4	Conformationally Defined Peptides as
6.3.2	Repair of Double-Stranded DNA Breaks in		Modulators of Protein Interactions 150
622	Mammalian Cells 104	7.4.1	Peptide Therapeutics 151
6.3.3	Meganucleases 105	7.5	Summary and Future Outlook 157
6.3.4	Zinc Finger Nucleases (ZFNs) 106		Questions 157
6.3.5	Transcription Activator–Like Effector		References 158
	Nucleases (TALENs) 108		
6.4	The Golden Age of Genome Engineering:	8	Protein Synthesis and
6.4	CRISPR-Based Genome Editing	8	Protein Synthesis and Engineering 167
	CRISPR-Based Genome Editing Technologies 108	8	
6.4.1	CRISPR-Based Genome Editing Technologies 108 Introduction 108	8 8.1	Engineering 167
6.4.1 6.4.2	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109		Engineering 167 Matthew R. Pratt and Tom W. Muir
6.4.1 6.4.2 6.4.3	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111	8.1	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167
6.4.1 6.4.2 6.4.3 6.4.4	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113	8.1 8.2	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 113	8.1 8.2	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 113 Precision Improvements 115	8.1 8.2 8.2.1	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6 6.4.7	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 113 Precision Improvements 115 Epigenome Editing 115	8.1 8.2 8.2.1	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 113 Precision Improvements 115 Epigenome Editing 115 Non-DSB-Reliant Genome Editing	8.1 8.2 8.2.1 8.2.1.1	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of N-terminal Cysteine Peptides 170
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6 6.4.7	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 113 Precision Improvements 115 Epigenome Editing 115 Non-DSB-Reliant Genome Editing Technologies 116	8.1 8.2 8.2.1 8.2.1.1	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of N-terminal Cysteine Peptides 170 C-terminal Pieces: Recombinant Expression
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6 6.4.7 6.5	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 113 Precision Improvements 115 Epigenome Editing 115 Non-DSB-Reliant Genome Editing Technologies 116 Base Editing 116	8.1 8.2 8.2.1 8.2.1.1	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of N-terminal Cysteine Peptides 170 C-terminal Pieces: Recombinant Expression of N-terminal Cysteine Peptides/
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6 6.4.7 6.5	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 113 Precision Improvements 115 Epigenome Editing 115 Non-DSB-Reliant Genome Editing Technologies 116 Base Editing 116 Prime Editing 119	8.1 8.2 8.2.1 8.2.1.1 8.2.1.2	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of N-terminal Cysteine Peptides 170 C-terminal Pieces: Recombinant Expression of N-terminal Cysteine Peptides/ Proteins 170 N-terminal Pieces: Chemical Synthesis of Thioester-Containing Peptides 171
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6 6.4.7 6.5	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 113 Precision Improvements 115 Epigenome Editing 115 Non-DSB-Reliant Genome Editing Technologies 116 Base Editing 116 Prime Editing 119 Gene Editing Methods for Spatial and	8.1 8.2 8.2.1 8.2.1.1 8.2.1.2	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of N-terminal Cysteine Peptides 170 C-terminal Pieces: Recombinant Expression of N-terminal Cysteine Peptides/ Proteins 170 N-terminal Pieces: Chemical Synthesis of
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6 6.4.7 6.5 6.5.1 6.5.2 6.6.6	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 113 Precision Improvements 115 Epigenome Editing 115 Non-DSB-Reliant Genome Editing Technologies 116 Base Editing 116 Prime Editing 119 Gene Editing Methods for Spatial and Temporal Control 119	8.1 8.2 8.2.1 8.2.1.1 8.2.1.2	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of N-terminal Cysteine Peptides 170 C-terminal Pieces: Recombinant Expression of N-terminal Cysteine Peptides/ Proteins 170 N-terminal Pieces: Chemical Synthesis of Thioester-Containing Peptides 171
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6 6.4.7 6.5	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 113 Precision Improvements 115 Epigenome Editing 115 Non-DSB-Reliant Genome Editing Technologies 116 Base Editing 116 Prime Editing 119 Gene Editing Methods for Spatial and Temporal Control 119 Ethical Implications, Summary, and Future	8.1 8.2 8.2.1 8.2.1.1 8.2.1.2	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of N-terminal Cysteine Peptides 170 C-terminal Pieces: Recombinant Expression of N-terminal Cysteine Peptides/ Proteins 170 N-terminal Pieces: Chemical Synthesis of Thioester-Containing Peptides 171 N-terminal Pieces: Recombinant Expression
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6 6.4.7 6.5 6.5.1 6.5.2 6.6.6	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 113 Precision Improvements 115 Epigenome Editing 115 Non-DSB-Reliant Genome Editing Technologies 116 Base Editing 116 Prime Editing 119 Gene Editing Methods for Spatial and Temporal Control 119 Ethical Implications, Summary, and Future Outlook 121	8.1 8.2 8.2.1 8.2.1.1 8.2.1.2 8.2.1.3 8.2.1.4	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of N-terminal Cysteine Peptides 170 C-terminal Pieces: Recombinant Expression of N-terminal Cysteine Peptides/ Proteins 170 N-terminal Pieces: Chemical Synthesis of Thioester-Containing Peptides 171 N-terminal Pieces: Recombinant Expression of Thioester-Containing Proteins 172
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6 6.4.7 6.5 6.5.1 6.5.2 6.6.6	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 113 Precision Improvements 115 Epigenome Editing 115 Non-DSB-Reliant Genome Editing Technologies 116 Base Editing 116 Prime Editing 119 Gene Editing Methods for Spatial and Temporal Control 119 Ethical Implications, Summary, and Future Outlook 121 Questions 123	8.1 8.2 8.2.1 8.2.1.1 8.2.1.2 8.2.1.3 8.2.1.4	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of N-terminal Cysteine Peptides 170 C-terminal Pieces: Recombinant Expression of N-terminal Cysteine Peptides/ Proteins 170 N-terminal Pieces: Chemical Synthesis of Thioester-Containing Peptides 171 N-terminal Pieces: Recombinant Expression of Thioester-Containing Proteins 172 Internal Fragments: Preparation of Cysteine
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6 6.4.7 6.5 6.5.1 6.5.2 6.6.6	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 113 Precision Improvements 115 Epigenome Editing 115 Non-DSB-Reliant Genome Editing Technologies 116 Base Editing 116 Prime Editing 119 Gene Editing Methods for Spatial and Temporal Control 119 Ethical Implications, Summary, and Future Outlook 121	8.1 8.2 8.2.1 8.2.1.1 8.2.1.2 8.2.1.3 8.2.1.4	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of N-terminal Cysteine Peptides 170 C-terminal Pieces: Recombinant Expression of N-terminal Cysteine Peptides/ Proteins 170 N-terminal Pieces: Chemical Synthesis of Thioester-Containing Peptides 171 N-terminal Pieces: Recombinant Expression of Thioester-Containing Proteins 172 Internal Fragments: Preparation of Cysteine and Thioester-Containing Peptides/
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6 6.4.7 6.5 6.5.1 6.5.2 6.6.6	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 113 Precision Improvements 115 Epigenome Editing 115 Non-DSB-Reliant Genome Editing Technologies 116 Base Editing 116 Prime Editing 119 Gene Editing Methods for Spatial and Temporal Control 119 Ethical Implications, Summary, and Future Outlook 121 Questions 123	8.1 8.2 8.2.1 8.2.1.1 8.2.1.2 8.2.1.3 8.2.1.4 8.2.1.5	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of N-terminal Cysteine Peptides 170 C-terminal Pieces: Recombinant Expression of N-terminal Cysteine Peptides/ Proteins 170 N-terminal Pieces: Chemical Synthesis of Thioester-Containing Peptides 171 N-terminal Pieces: Recombinant Expression of Thioester-Containing Proteins 172 Internal Fragments: Preparation of Cysteine and Thioester-Containing Peptides/ Proteins 172
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6 6.4.7 6.5 6.5.1 6.5.2 6.6.6	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 113 Precision Improvements 115 Epigenome Editing 115 Non-DSB-Reliant Genome Editing Technologies 116 Base Editing 116 Prime Editing 119 Gene Editing Methods for Spatial and Temporal Control 119 Ethical Implications, Summary, and Future Outlook 121 Questions 123	8.1 8.2 8.2.1 8.2.1.1 8.2.1.2 8.2.1.3 8.2.1.4 8.2.1.5	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of N-terminal Cysteine Peptides 170 C-terminal Pieces: Recombinant Expression of N-terminal Cysteine Peptides/ Proteins 170 N-terminal Pieces: Chemical Synthesis of Thioester-Containing Peptides 171 N-terminal Pieces: Recombinant Expression of Thioester-Containing Proteins 172 Internal Fragments: Preparation of Cysteine and Thioester-Containing Peptides/ Proteins 172 Adding More Pieces: Moving Beyond
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6 6.4.7 6.5 6.5.1 6.5.2 6.6	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 115 Epigenome Editing 115 Non-DSB-Reliant Genome Editing Technologies 116 Base Editing 119 Gene Editing 119 Gene Editing Methods for Spatial and Temporal Control 119 Ethical Implications, Summary, and Future Outlook 121 Questions 123 References 123 Peptide Synthesis and Engineering 135	8.1 8.2 8.2.1 8.2.1.1 8.2.1.2 8.2.1.3 8.2.1.4 8.2.1.5	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of N-terminal Cysteine Peptides 170 C-terminal Pieces: Recombinant Expression of N-terminal Cysteine Peptides/ Proteins 170 N-terminal Pieces: Chemical Synthesis of Thioester-Containing Peptides 171 N-terminal Pieces: Recombinant Expression of Thioester-Containing Proteins 172 Internal Fragments: Preparation of Cysteine and Thioester-Containing Peptides/ Proteins 172 Adding More Pieces: Moving Beyond Thioester/Cysteine Ligations 173
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6 6.4.7 6.5 6.5.1 6.5.2 6.6	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 115 Epigenome Editing 115 Non-DSB-Reliant Genome Editing Technologies 116 Base Editing 116 Prime Editing 119 Gene Editing Methods for Spatial and Temporal Control 119 Ethical Implications, Summary, and Future Outlook 121 Questions 123 References 123 Peptide Synthesis and	8.1 8.2 8.2.1 8.2.1.1 8.2.1.2 8.2.1.3 8.2.1.4 8.2.1.5 8.2.2 8.2.2.1	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of N-terminal Cysteine Peptides 170 C-terminal Pieces: Recombinant Expression of N-terminal Cysteine Peptides/ Proteins 170 N-terminal Pieces: Chemical Synthesis of Thioester-Containing Peptides 171 N-terminal Pieces: Recombinant Expression of Thioester-Containing Proteins 172 Internal Fragments: Preparation of Cysteine and Thioester-Containing Peptides/ Proteins 172 Adding More Pieces: Moving Beyond Thioester/Cysteine Ligations 173 Desulfurization 174 Auxiliaries 174 Other Ligation Chemistries 176
6.4.1 6.4.2 6.4.3 6.4.4 6.4.5 6.4.6 6.4.7 6.5 6.5.1 6.5.2 6.6	CRISPR-Based Genome Editing Technologies 108 Introduction 108 CRISPR-Cas9 109 Programmability Improvements 111 Efficiency Improvements 113 Specificity Improvements 115 Epigenome Editing 115 Non-DSB-Reliant Genome Editing Technologies 116 Base Editing 119 Gene Editing 119 Gene Editing Methods for Spatial and Temporal Control 119 Ethical Implications, Summary, and Future Outlook 121 Questions 123 References 123 Peptide Synthesis and Engineering 135	8.1 8.2 8.2.1.1 8.2.1.2 8.2.1.3 8.2.1.4 8.2.1.5 8.2.2.1 8.2.2.1 8.2.2.2	Engineering 167 Matthew R. Pratt and Tom W. Muir Introduction/Historical Perspective 167 Core Concepts/Landmark Studies 170 Cysteine-thioester-Based Ligations: Making the Pieces 170 C-terminal Pieces: Chemical Synthesis of N-terminal Cysteine Peptides 170 C-terminal Pieces: Recombinant Expression of N-terminal Cysteine Peptides/ Proteins 170 N-terminal Pieces: Chemical Synthesis of Thioester-Containing Peptides 171 N-terminal Pieces: Recombinant Expression of Thioester-Containing Proteins 172 Internal Fragments: Preparation of Cysteine and Thioester-Containing Peptides/ Proteins 172 Adding More Pieces: Moving Beyond Thioester/Cysteine Ligations 173 Desulfurization 174 Auxiliaries 174

viii	Contents		
		_	

8.4 8.5	Protein Trans-splicing 178	9.3.6	A Multi-Functional Genome-Wide CRISPR
	Examples of Protein Synthesis 179	0.4	System 212
8.5.1	Post-Translational Modifications 179	9.4	Future Perspectives and Conclusion 212
8.5.1.1	Cell Signaling 179		Acknowledgments 213
8.5.1.2	Chromatin 181		Questions 213
8.5.1.3	Amyloid-Forming Proteins 181		References 214
8.5.2	Chemical and Biophysical Probes 182		
8.5.2.1	Backbone Modifications 182	10	Chemical Biology of Cellular
8.5.2.2	Segmental Isotopic Labeling 182		Metabolism 221
8.5.3	Mirror Image Proteins 184		Peter C. Gray and Alan Saghatelian
8.5.3.1	Racemic Crystallography 184	10.1	Introduction/Historical Perspective 221
8.5.3.2	Mirror Image Display 184	10.2	Metabolite Detection and Quantitation 223
8.5.4	Protein Ligation in Living Systems 184	10.2.1	Shotgun Metabolomics 223
8.5.5	Potential Therapeutic Applications 184	10.2.2	Targeted Metabolomics 225
8.6	Summary and Future Outlook 185	10.2.3	Metabolite Flux Analysis 225
	Questions 185	10.2.4	Untargeted Metabolomics 227
	References 186	10.2.5	Discovering Structurally Novel
			Metabolites 228
9	Directed Evolution for Chemical	10.3	Metabolite Imaging and Sensing 229
	Biology 193	10.3.1	Mass Spectrometry Imaging 229
	Pu Xue, Fang Guo, Linzixuan Zhang, and	10.3.2	Chemical Probes for Metabolite
	Huimin Zhao		Imaging 230
9.1	Introduction 193	10.3.3	Protein and RNA Metabolite Sensors 232
9.2	Methodologies 195	10.4	Perturbation of Metabolite Levels 234
9.2.1	Directed Evolution at the Protein Level 195	10.4.1	Small-Molecule Inhibitors and Drugs of
9.2.1.1	Random Mutagenesis 195		Metabolism 234
9.2.1.2	Gene Recombination 195	10.4.2	Enzymatic Perturbation of Metabolism 235
9.2.1.3	Semi-Rational Design 197	10.5	The Impact of Chemical Biology in Disease
9.2.2	Directed Evolution at the Pathway Level 198		and Drug Discovery 236
9.2.2.1	Directed Evolution of a Single Enzyme in	10.6	Summary and Future Outlook 237
	a Pathway 198		Questions 238
9.2.2.2	Directed Evolution of an Entire		References 238
	Pathway 199		
9.2.3	Directed Evolution at the Genome	11	Chemical Biology of Lipids 243
	Level 199		Scotland Farley, Alix Thomas, Aurélien Laguerre,
9.2.3.1	Adaptive Laboratory Evolution 199		and Carsten Schultz
9.2.3.2	Genome-Scale Engineering Strategies 200	11.1	Introduction 243
9.2.4	Continuous Directed Evolution 200	11.2	Identification of Bulk Lipids 245
9.2.5	Screening or Selection Methods 201	11.2.1	Lipidomics by Mass Spectrometry 245
9.2.5.1	Selection-Based Techniques 202	11.2.2	Lipid Analysis by Thin-Layer
9.2.5.2	Screening-Based Techniques 203		Chromatography 247
9.3	Case Studies 203	11.3	Fixing Lipids in Subcellular Space 247
9.3.1	Directed Evolution of a Glyphosate	11.3.1	Protein-Based Techniques to Localize
	N-Acetyltransferase 203		Lipids 248
9.3.2	Directed Evolution of a Transaminase for	11.3.2	Mass Spectrometry Imaging of Lipids 249
	Sitagliptin Manufacture 207	11.3.3	Lipid Detection Using Modified Lipids as
9.3.3	Directed Evolution of a Cytokine Using DNA		Probes 249
	Family Shuffling 208	11.4	Tracing Individual Lipids via In Cellulo Click
9.3.4	Efficient Proximity Labeling in Living Cells		Chemistry 250
	and Organisms with TurboID 210	11.4.1	Alkyne/Azide-Modified Lipids and Click
9.3.5	Biocatalytic Cascade Evolution for		Chemistry 251
	Manufacturing Islatravir 211	11.4.2	Bifunctional Lipid Derivatives 251

11.5	Tools to Elucidate Lipid Signaling 253	13	Chemical Glycobiology 295
11.5.1	Metabolic Machinery as a Chemical Tool:		Amélie M. Joffrin, Alexander W. Sorum, and
	the Advantage of Chemical Dimerizers 253		Linda C. Hsieh-Wilson
11.5.2	Releasing Bioactive Lipids with Light 255	13.1	Introduction 295
11.6	A Comprehensive View of Protein-Lipid	13.2	Total Chemical Synthesis of
	Interactions 256		StructurallyDefined Glycans 297
11.6.1	Trifunctional Lipids 256	13.3	Enzymatic and Chemoenzymatic Synthesis of
11.6.2	Lipid-Protein Interactome 258		Glycans 300
11.7	Summary and Future Outlook 258	13.4	Programmable and Automated Glycan
	Questions 259		Synthesis 302
	References 259	13.5	Synthesis of Glycopeptides and
	10101011005		Glycoproteins 303
		13.6	Glycan Microarrays 305
12	Protein Posttranslational	13.7	Chemical Tagging and Remodeling of Cellular
	Modifications 267		Glycans 306
	Sam Whedon and Philip A. Cole	13.8	Inhibitors of Glycan-Processing Enzymes and
12.1	Introduction 267		Glycan Binding Proteins 310
12.2	Functional Impacts of PTMs 268	13.9	Glycan-Targeted Therapeutics 313
12.3	Evolution and PTMs 270	13.10	Summary and Future Outlook 316
12.4	Major Classes of PTMs 270	10.10	Questions 317
12.4.1	Phosphorylation 270		References 317
12.4.2	Acetylation 272		northings bir,
12.4.3	Ubiquitination 273	14	The Chemical and Enzymatic
12.4.4	Methylation 275	• '	Modification of Proteins 329
12.4.5	Glycosylation 275		Nicholas S. Dolan, Johnathan C. Maza,
12.4.6	Lipidation of Proteins 278		Alexandra V. Ramsey, and Matthew B. Francis
12.4.7	Oxidation of Proteins 278	14.1	Introduction 329
12.4.8	Miscellaneous Modifications 278	14.2	General Considerations 329
12.5	Writers and Erasers 280	14.3	Lysine Modification 330
12.5.1	Protein Kinases and Phosphatases 280	14.4	Aspartic Acid, Glutamic Acid, and
12.5.2	Acetyltransferases and Deacetylases 280	1	C-Terminal Carboxylate Modification 333
12.5.3	Ubiquitin Ligases and	14.5	Tyrosine Modification 334
	Deubiquitinases 281	14.6	Cysteine Modification 337
12.5.4	Methylation and Demethylases 281	14.7	Methionine Modification 340
12.5.5	Glycosyltransferases and	14.8	Tryptophan Modification 341
	Glycosidases 282	14.9	Histidine Modification 343
12.5.6	Lipid Transferase and Hydrolases 282	14.10	Serine and Threonine Modification 344
12.6	Strategies for the Study of PTMs 283	14.11	N-Terminal Modification 344
12.6.1	Mutagenesis 283	14.12	Enzymatic Approaches to Modifying
12.6.2	Genetic Codon Expansion 283	1	Proteins 347
12.6.3	Small-Molecule Probes and Chemical	14.12.1	Transpeptidases 347
12.0.0	Complementarity 283	14.12.2	Ligases 348
12.6.4	Chemical Ligation 284	14.12.3	Activating Enzymes 349
12.6.5	Protein Microarrays 284	14.13	Summary and Future Outlook 350
12.7	Protein PTMs in Diseases 284	14.15	Questions 350
12.7.1	Protein Kinases and Diseases 285		References 352
12.7.2	Lys Acetylation and Cutaneous T Cell		References 332
	Lymphoma 285	15	Genetic Code Expansion 359
12.7.3	Ubiquitination 285	13	Peng R. Chen, Shixian Lin, and Jie P. Li
12.7.3	Summary 286	15.1	Introduction 359
	Questions 286	15.1	Genetic Code Expansion Through Directed
	References 287	13.4	Evolution of aaRS/tRNA Pairs 359
			Living of animal and and and animal a

15.2.1	The Development of the Mj.TyrRS-tRNA Based GCE System 360	16.6	Cu-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) of Azides and Terminal
15.2.2	The Development of Additional	16.7	Alkynes 393
15.2.3	aaRS/tRNA-Based GCE System 362 The PylRS-tRNA Pair as a "one-stop-shop"	16.7	Strain-Promoted [3+2] Azide–Alkyne Cycloaddition (SPAAC) of Azides and
	GCE System 362	16.0	Cyclooctynes 395
15.2.4	Genetic Code Expansion in Multicellular	16.8	The Tetrazine Ligation: Rapid Bioorthogonal
15.3	Organisms 363		Inverse Electron-Demand Diels-Alder Reactions 396
15.3	GCE with Genome Recoding Strains and/or	16.9	Other Bioorthogonal Ligations 398
1521	Unnatural Codons 364	16.10	Light-Activated Bioorthogonal
15.3.1	GCE with Genome Recoding Strains 364	10.10	Reactions 399
15.3.2	GCE with Four-Base Codons Using	16.11	Bioorthogonal Uncaging and Cleavage
1522	Orthogonal Ribosome 366	10.11	Reactions 400
15.3.3	Genetic Code Expansion with Unnatural Base	16.12	Mutually Orthogonal Bioorthogonal
15.4	Pairs 366	10.12	Reactions 401
15.4	GCE-based Applications 366	16.13	Fluorogenic Bioorthogonal Reagents 402
15.4.1	Site-Specific Posttranslational Modifications	16.14	Applications of Bioorthogonal
15.40	(PTMs) 367	10.17	Chemistry 403
15.4.2	New "Physical" Property Empowered by	16.14.1	-
15.43	ncAAs 369	10.14.1	Tagging (BONCAT) 403
15.4.3	New Chemical Reactivity Derived from ncAA	16.14.2	
1544	and Their Unique Applications 369	16.14.3	Therapeutic Applications of Bioorthogonal
15.4.4	Control of Protein Activation 372	10.14.5	Chemistry 404
15.5	Therapeutic Conjugates 374	16.15	Summary and Future Outlook 406
15.6	Live-Attenuated Virus and Other Genetically	20120	Questions 406
157	Modified Vaccines 375		References 407
15.7 15.7.1	Summary and Future Outlook 376 Improving the Efficiency 376		
15.7.1	Expanding the Applications 376	17	Cellular Imaging 415
15.7.2	Exploring the Therapeutic Potential 377		Amy E. Palmer and Luke D. Lavis
13.7.3	Questions 377	17.1	Introduction 415
	References 377	17.1.1	History 415
	References 377	17.1.2	Light and Fluorescence 417
		17.2	Small-Molecule Fluorophores 417
16	Bioorthogonal Chemistry 387	17.2.1	Background 417
	Jeremy Baskin and Pamela Chang	17.2.2	Pyrenes and Coumarin Fluorophores 417
16.1	Introduction and Historical	17.2.3	BODIPY Dyes 418
	Perspective 387	17.2.4	Fluoresceins and Rhodamines 418
16.2	Key Concepts: Bioorthogonality and	17.2.5	Phenoxazine and Cyanine Dyes 419
	Bioorthogonal Reactions, Click Chemistry,	17.2.6	Use as Biomolecule Labels 419
	and the Bioorthogonal Metabolic Reporter	17.2.7	Use as Cellular Stains 419
	Strategy 388	17.2.8	Fluorescent Indicators 420
16.2.1	Bioorthogonality and Bioorthogonal	17.2.9	Enzyme Substrates 421
	Reactions 388	17.3	Fluorescent Proteins 421
16.2.2	Click Chemistry 388	17.3.1	Background 421
16.2.3	The Bioorthogonal Metabolic Reporter	17.3.2	General Considerations of Fluorescent
	Strategy 389		Proteins 421
16.3	The Beginnings of Bioorthogonal Chemistry:	17.3.3	Fluorescent Proteins as Biomolecule Labels
	Oxime and Hydrazone Formation 390		and "Stains" 422
16.4	The Azide as a Bioorthogonal Handle 391	17.3.4	Fluorescent Proteins as Sensors 423
16.5	The Staudinger Ligation of Azides and	17.3.5	Fluorescent Proteins as Enzyme
	Phosphines 392		Substrates 423

17.4	Hybrid Small-Molecule-Protein	19	Chemical Biology of Metals 459
	Systems 424		Eva J. Ge, Patricia De La Torre, and
17.4.1	Background 424		Christopher J. Chang
17.4.2	Labels 425	19.1	Introduction 459
17.4.3	Sensors 426	19.2	Metals and the Inorganic Foundations of
17.5	Landmark Study I: Harnessing Photosensitive		Life 459
	Fluorophores 426	19.2.1	Metal Complexes are Lewis Acid-Base
17.5.1	Background 426		Complexes 459
17.5.2	Super-Resolution Microscopy 426	19.2.2	Crystal Field Theory Enables Bonding
17.6	Landmark Study II: Ca ²⁺ Imaging 427		Analysis from Molecular Shape and d
17.6.1	Background 427		Orbitals 460
17.6.2	Small-Molecule Ca ²⁺ Indicators 427	19.2.3	Hard Soft Acid Base Theory Defines
17.6.3	Genetically Encoded Ca ²⁺		Metal-Ligand Preferences 462
	Indicators 428	19.3	Non-Redox Roles for Metals in Biology:
17.6.4	Genetically Encoded Indicators for In Vivo	17.5	Structure and Lewis Acid Catalysis 462
	Imaging 428	19.3.1	Metals for Stabilizing Nucleic Acid
17.7	Summary and Future Outlook 430	19.5.1	Structure 463
	Questions 430	19.3.2	Metals as Protein Structural Units: Zinc
	References 430	19.3.2	
		10 2 2	Finger and EF Hand Motifs 463
18	<i>In Vivo</i> Imaging 435	19.3.3	Metals as Lewis Acid Catalysts:
	Zi Yao and Jennifer A. Prescher	10.4	Metallohydrolases 464
18.1	Introduction 435	19.4	Redox Chemistry: Oxygen Transport and
18.2	Basic Concepts for Imaging In Vivo 436		Electron Transfer Proteins 464
18.3	The Imaging Toolbox: Probes for Imaging	19.4.1	Oxygen Transport Requires Redox-Active
	Cellular and Molecular Features 438		Metal Binding 465
18.3.1	"Always On" Probes 439	19.4.2	Marcus Theory and Electron Transfer
18.3.2	"Turn-On" (Activatable) Probes 439		Proteins 465
18.3.3	Genetically Encoded Probes 439	19.5	Redox Chemistry: Metalloenzymes for Redox
18.4	Molecular Imaging Across the		Catalysis at Oxygen, Nitrogen, and
10	Electromagnetic Spectrum 440		Carbon 467
18.4.1	Imaging with X-rays (CT) 440	19.5.1	Oxygen Evolution in Photosynthesis:
18.4.2	Imaging with Sound (US) 440		Photosystem II 467
18.4.3	Imaging with Radio Waves (MRI) 441	19.5.2	Oxygen Reduction: Respiration with
18.4.4	Imaging with Radionuclides		Cytochrome c Oxidase 467
10	(PET/SPECT) 442	19.5.3	Oxygen Catalysis: Heme and Non-Heme
18.4.5	Imaging with Optical Light		Iron-Dependent Oxidations 468
101.10	(Fluorescence/Bioluminescence) 444	19.5.4	Nitrogen Cycle: Nitrogenases and
18.4.5.1	Targeted Fluorophores and Fluorescent		Nitrate/Nitrite Reductases 469
20111012	Materials 445	19.5.5	Bioorganometallic Chemistry: Carbon
18.4.5.2	Activatable Probes 445		Cycling and Vitamin B12 469
	Genetically Encoded Fluorescent Probes 445	19.6	Metals in Medicine: Metallotargets,
	Genetically Encoded Bioluminescent Proteins		Metallodrugs, and Metal-Based Imaging
	(Luciferases) 447		Agents 470
18.4.5.5	Engineered Probes for Sensing Metabolites	19.7	Emerging Areas for Metals in Biology:
251 11212	and Molecular Features 447		Transition Metal Signaling and
18.5	Multimodality Imaging and Combination		Metalloallostery 472
	Probes 449	19.8	Chemical Tools to Study Metal
18.6	Emerging Areas in Molecular Imaging 450		Biology 472
18.7	Summary and Future Outlook 450	19.9	Summary and Future Outlook 475
	Questions 451		Questions 475
	References 451		References 476

20	Redox Chemical Biology 481 Yunlong Shi and Kate S. Carroll	21.3	Summary and Future Outlook 518 Questions 518
20.1	Introduction 481		References 519
20.2	Activity-Based Detection of Cysteine		References 319
20.2	Modifications 484	22	Chamical Canadian 200
20.3	Indirect Profiling of Cysteine Oxidation 484	22	Chemical Genetics 527 Michael S. Cohen
20.4	Direct Profiling of Cysteine Oxidation 404	22.1	
20.4	Chemoselective Probes 486	22.1	Introduction 527
20.4.1	5 011 5 4 5 40 4 5 4 4 5 5 5 5 5 5 5 5 5 5 5	22.2	AS-Protein – Orthogonal Molecular
	Profiling Protein Sulfenic Acids (—SOH) 486 Sulfenic Acid Probes – A Historical	22.2	Glues 528
20.4.1.1	Perspective 486	22.3	AS-Enzyme – Orthogonal Substrate
20 4 1 2	Chemical Models for the Assessment of	22.3.1	Pairs 532 Protein Kinases 532
20.7.1.2	Sulfenic Acid Probes 486		
20 4 1 3	Selectivity of Chemical Probes for Sulfenic	22.3.2	Protein Methyltransferases 535
20.4.1.3	Acids 486	22.3.3	Protein Lysine Acetyltransferases 538
20.4.1.4	Quantification of Protein Sulfenic Acids 489	22.3.4	PARPs 539
	Application of Sulfenic Acid Probes 492	22.3.5	Glycosyltransferases 542
20.4.1.3	Profiling Protein Sulfinic Acids	22.3.6	PTM Erasers: Lysine Demethylases 544
20.4.2	(-SO ₂ H) 492	22.3.7	Beyond PTM Enzymes 544
20.4.3	Profiling Protein Persulfides (–SSH) 493	22.4	AS-Enzyme – Orthogonal Inhibitor
20.4.3	Probes and Biosensors for Reactive Oxygen	22.4.1	Pairs 547
20.3	Species in Cells 494	22.4.1	Protein Kinases 547
20.6	Conclusions and Outlook 496	22.4.2	Other Enzymes 549
20.0	Questions 496	22.5	Final Thoughts 549
	References 497	22.5.1	Beyond Bump-Hole 549
	References 497		Questions 550
21	Activity Pagad Protain		References 550
21	Activity-Based Protein Profiling 503	77	National Document Discourses 555
	William H. Parsons and Benjamin F. Cravatt	23	Natural Product Discovery 555
21.1	Introduction/Historical Perspective 503		Mohammad R. Seyedsayamdost, Brett C.
21.2	Core Concepts/Landmark Studies 504	23.1	Covington, Yifan Zhang, and Yuchen Li Introduction and Definitions 555
21.2.1	Probe Design 504	23.1	Key Concept: Natural Products are
	· ·	23.2	
			Constiguilly Engeded 556
	Reactive Groups 504	22.2	Genetically Encoded 556
	Reporter Tags 507	23.3	Key Concept: Structural Differences Between
21.2.1.3	Reporter Tags 507 Recognition Group/Linker 508		Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558
21.2.1.3 21.2.2	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509	23.3 23.4	Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent
21.2.1.3 21.2.2 21.2.2.1	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509 Gel-Based Analysis 509	23.4	Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent Reactivity 559
21.2.1.3 21.2.2 21.2.2.1 21.2.2.2	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509 Gel-Based Analysis 509 Fluorescence Polarization 510		Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent Reactivity 559 Key Concept: Natural Product Discovery and
21.2.1.3 21.2.2 21.2.2.1 21.2.2.2	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509 Gel-Based Analysis 509 Fluorescence Polarization 510 Imaging of Proteins in Cells and	23.4 23.5	Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent Reactivity 559 Key Concept: Natural Product Discovery and Activity-Guided Fractionation 561
21.2.1.3 21.2.2 21.2.2.1 21.2.2.2 21.2.2.3	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509 Gel-Based Analysis 509 Fluorescence Polarization 510 Imaging of Proteins in Cells and Organisms 511	23.4	Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent Reactivity 559 Key Concept: Natural Product Discovery and Activity-Guided Fractionation 561 Key Concept: Cryptic Biosynthetic Gene
21.2.1.3 21.2.2 21.2.2.1 21.2.2.2 21.2.2.3	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509 Gel-Based Analysis 509 Fluorescence Polarization 510 Imaging of Proteins in Cells and Organisms 511 Quantitative Proteomics by Mass	23.4 23.5 23.6	Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent Reactivity 559 Key Concept: Natural Product Discovery and Activity-Guided Fractionation 561 Key Concept: Cryptic Biosynthetic Gene Clusters 562
21.2.1.3 21.2.2 21.2.2.1 21.2.2.2 21.2.2.3 21.2.2.4	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509 Gel-Based Analysis 509 Fluorescence Polarization 510 Imaging of Proteins in Cells and Organisms 511 Quantitative Proteomics by Mass Spectrometry 511	23.4 23.5	Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent Reactivity 559 Key Concept: Natural Product Discovery and Activity-Guided Fractionation 561 Key Concept: Cryptic Biosynthetic Gene Clusters 562 Landmark Studies: Penicillin and the Golden
21.2.1.3 21.2.2 21.2.2.1 21.2.2.2 21.2.2.3 21.2.2.4 21.2.3	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509 Gel-Based Analysis 509 Fluorescence Polarization 510 Imaging of Proteins in Cells and Organisms 511 Quantitative Proteomics by Mass Spectrometry 511 Common Applications 512	23.423.523.623.7	Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent Reactivity 559 Key Concept: Natural Product Discovery and Activity-Guided Fractionation 561 Key Concept: Cryptic Biosynthetic Gene Clusters 562 Landmark Studies: Penicillin and the Golden Age of Antibiotic Discovery 563
21.2.1.3 21.2.2 21.2.2.1 21.2.2.2 21.2.2.3 21.2.2.4 21.2.3	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509 Gel-Based Analysis 509 Fluorescence Polarization 510 Imaging of Proteins in Cells and Organisms 511 Quantitative Proteomics by Mass Spectrometry 511 Common Applications 512 Profiling Protein Activity and Amino Acid	23.4 23.5 23.6	Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent Reactivity 559 Key Concept: Natural Product Discovery and Activity-Guided Fractionation 561 Key Concept: Cryptic Biosynthetic Gene Clusters 562 Landmark Studies: Penicillin and the Golden Age of Antibiotic Discovery 563 Landmark Studies: Activating Silent
21.2.1.3 21.2.2 21.2.2.1 21.2.2.2 21.2.2.3 21.2.2.4 21.2.3	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509 Gel-Based Analysis 509 Fluorescence Polarization 510 Imaging of Proteins in Cells and Organisms 511 Quantitative Proteomics by Mass Spectrometry 511 Common Applications 512 Profiling Protein Activity and Amino Acid Reactivity in Biological Systems of	23.4 23.5 23.6 23.7 23.8	Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent Reactivity 559 Key Concept: Natural Product Discovery and Activity-Guided Fractionation 561 Key Concept: Cryptic Biosynthetic Gene Clusters 562 Landmark Studies: Penicillin and the Golden Age of Antibiotic Discovery 563 Landmark Studies: Activating Silent Biosynthetic Gene Clusters 565
21.2.1.3 21.2.2 21.2.2.1 21.2.2.2 21.2.2.3 21.2.2.4 21.2.3 21.2.3.1	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509 Gel-Based Analysis 509 Fluorescence Polarization 510 Imaging of Proteins in Cells and Organisms 511 Quantitative Proteomics by Mass Spectrometry 511 Common Applications 512 Profiling Protein Activity and Amino Acid Reactivity in Biological Systems of Interest 513	23.4 23.5 23.6 23.7 23.8 23.8.1	Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent Reactivity 559 Key Concept: Natural Product Discovery and Activity-Guided Fractionation 561 Key Concept: Cryptic Biosynthetic Gene Clusters 562 Landmark Studies: Penicillin and the Golden Age of Antibiotic Discovery 563 Landmark Studies: Activating Silent Biosynthetic Gene Clusters 565 Manipulation of Culture Conditions 565
21.2.1.3 21.2.2 21.2.2.1 21.2.2.2 21.2.2.3 21.2.2.4 21.2.3 21.2.3.1	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509 Gel-Based Analysis 509 Fluorescence Polarization 510 Imaging of Proteins in Cells and Organisms 511 Quantitative Proteomics by Mass Spectrometry 511 Common Applications 512 Profiling Protein Activity and Amino Acid Reactivity in Biological Systems of Interest 513 Competitive ABPP for Ligand Discovery and	23.4 23.5 23.6 23.7 23.8 23.8.1 23.8.2	Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent Reactivity 559 Key Concept: Natural Product Discovery and Activity-Guided Fractionation 561 Key Concept: Cryptic Biosynthetic Gene Clusters 562 Landmark Studies: Penicillin and the Golden Age of Antibiotic Discovery 563 Landmark Studies: Activating Silent Biosynthetic Gene Clusters 565 Manipulation of Culture Conditions 565 Classical Genetics 566
21.2.1.3 21.2.2 21.2.2.1 21.2.2.2 21.2.2.3 21.2.2.4 21.2.3 21.2.3.1	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509 Gel-Based Analysis 509 Fluorescence Polarization 510 Imaging of Proteins in Cells and Organisms 511 Quantitative Proteomics by Mass Spectrometry 511 Common Applications 512 Profiling Protein Activity and Amino Acid Reactivity in Biological Systems of Interest 513 Competitive ABPP for Ligand Discovery and Optimization 513	23.4 23.5 23.6 23.7 23.8 23.8.1 23.8.2 23.8.3	Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent Reactivity 559 Key Concept: Natural Product Discovery and Activity-Guided Fractionation 561 Key Concept: Cryptic Biosynthetic Gene Clusters 562 Landmark Studies: Penicillin and the Golden Age of Antibiotic Discovery 563 Landmark Studies: Activating Silent Biosynthetic Gene Clusters 565 Manipulation of Culture Conditions 565 Classical Genetics 566 Chemical Genetics 566
21.2.1.3 21.2.2 21.2.2.1 21.2.2.2 21.2.2.3 21.2.2.4 21.2.3 21.2.3.1 21.2.3.2	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509 Gel-Based Analysis 509 Fluorescence Polarization 510 Imaging of Proteins in Cells and Organisms 511 Quantitative Proteomics by Mass Spectrometry 511 Common Applications 512 Profiling Protein Activity and Amino Acid Reactivity in Biological Systems of Interest 513 Competitive ABPP for Ligand Discovery and Optimization 513 Target Identification for Ligands 515	23.4 23.5 23.6 23.7 23.8 23.8.1 23.8.2 23.8.3 23.8.4	Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent Reactivity 559 Key Concept: Natural Product Discovery and Activity-Guided Fractionation 561 Key Concept: Cryptic Biosynthetic Gene Clusters 562 Landmark Studies: Penicillin and the Golden Age of Antibiotic Discovery 563 Landmark Studies: Activating Silent Biosynthetic Gene Clusters 565 Manipulation of Culture Conditions 565 Classical Genetics 566 Chemical Genetics 567 Heterologous Expression 568
21.2.1.3 21.2.2 21.2.2.1 21.2.2.2 21.2.2.3 21.2.3.1 21.2.3.2 21.2.3.2 21.2.3.3 21.2.3.4	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509 Gel-Based Analysis 509 Fluorescence Polarization 510 Imaging of Proteins in Cells and Organisms 511 Quantitative Proteomics by Mass Spectrometry 511 Common Applications 512 Profiling Protein Activity and Amino Acid Reactivity in Biological Systems of Interest 513 Competitive ABPP for Ligand Discovery and Optimization 513 Target Identification for Ligands 515 Assignment of Enzyme Function 516	23.4 23.5 23.6 23.7 23.8 23.8.1 23.8.2 23.8.3	Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent Reactivity 559 Key Concept: Natural Product Discovery and Activity-Guided Fractionation 561 Key Concept: Cryptic Biosynthetic Gene Clusters 562 Landmark Studies: Penicillin and the Golden Age of Antibiotic Discovery 563 Landmark Studies: Activating Silent Biosynthetic Gene Clusters 565 Manipulation of Culture Conditions 565 Classical Genetics 566 Chemical Genetics 567 Heterologous Expression 568 Summary and Outlook 569
21.2.1.3 21.2.2 21.2.2.1 21.2.2.2 21.2.2.3 21.2.3.1 21.2.3.2 21.2.3.2 21.2.3.3 21.2.3.4	Reporter Tags 507 Recognition Group/Linker 508 Detection Methods 509 Gel-Based Analysis 509 Fluorescence Polarization 510 Imaging of Proteins in Cells and Organisms 511 Quantitative Proteomics by Mass Spectrometry 511 Common Applications 512 Profiling Protein Activity and Amino Acid Reactivity in Biological Systems of Interest 513 Competitive ABPP for Ligand Discovery and Optimization 513 Target Identification for Ligands 515	23.4 23.5 23.6 23.7 23.8 23.8.1 23.8.2 23.8.3 23.8.4	Key Concept: Structural Differences Between Natural Products and Synthetic Drugs 558 Key Concept: Target Specificity and Latent Reactivity 559 Key Concept: Natural Product Discovery and Activity-Guided Fractionation 561 Key Concept: Cryptic Biosynthetic Gene Clusters 562 Landmark Studies: Penicillin and the Golden Age of Antibiotic Discovery 563 Landmark Studies: Activating Silent Biosynthetic Gene Clusters 565 Manipulation of Culture Conditions 565 Classical Genetics 566 Chemical Genetics 567 Heterologous Expression 568

24	Natural Product Biosynthesis 575	25.5.2	Substrate Analogue PG Probes 612
	Eun Bin Go and Yi Tang	25.6	Labeling Glycan Cell Envelope
24.1	Introduction 575		Components 613
24.2	Peptide Natural Products 577	25.6.1	Diversity and Function of Bacterial
24.2.1	Ribosomally Synthesized and		Polysaccharides 613
	Post-translationally Modified Peptides	25.6.2	Probes of Bacterial Glycans 614
	(RiPPs) 577	25.6.3	Probes of LPS: AzKdo 615
24.2.2	Non-ribosomal Peptides 579	25.6.4	Labeling Mycobacterial Glycans 615
24.3	Polyketide Natural Products 582	25.6.5	Trehalose Analogs 616
24.3.1	Bacterial Type-I Polyketides 584	25.6.6	Imaging Probes 616
24.3.2	Bacterial Type-II Polyketides 586	25.6.7	Fluorogenic Probes 617
24.4	Terpene Natural Products 588	25.7	Chemical Probes Applied to the
24.5	Hybrid and Unnatural Natural Products 591		Microbiome 617
24.6	Summary and Future Outlook 592	25.7.1	Microbiome: Looking Forward 618
	Acknowledgment 592	25.8	Summary and Future Outlook 619
	Questions 593		Questions 619
	References 594		References 620
25	Chemical Microbiology 597	26	Chemical Approaches to Analyze
	Victoria M. Marando, Stephanie R. Smelyansky,		Biological Mechanisms and
	Daria E. Kim, and Laura L. Kiessling		Overcome Resistance to
25.1	Introduction and History 597		Therapeutics 629
25.2	Cell Envelope Structure and		Rudolf Pisa, Tommaso Cupido, and
	Biosynthesis 598		Tarun M. Kapoor
25.2.1	Bacterial Cell Structure 598	26.1	Introduction 629
25.3	Chemical and Chemoenzymatic Synthesis for	26.2	Using Chemical Inhibitors as Tools to Probe
	Pathway Elucidation 600		Cellular Processes 630
25.3.1	Peptidoglycan (PG) Biosynthesis 600	26.3	Using Resistance to Characterize Chemical
	Reconstructing the Steps in PG Biosynthesis		Inhibitors 632
	Using Defined Substrates 600	26.4	Crash-Testing Drugs 633
25.3.1.2	Accessing Lipids I and II 602	26.5	RADD - Resistance Analysis During
25.3.2	Cell Envelope Components Beyond		Design 635
	Peptidoglycan 603	26.6	Designing Inhibitors with Distinct Binding
25.3.2.1	Gram-Negative Lipopolysaccharides 603		Modes 636
	Wall Teichoic Acid Biosynthesis 605	26.7	Addressing Drug Resistance with Targeted
	Mycobacterial Galactan 605		Protein Degradation 639
25.4	The Chemical Biology of Antibiotic	26.8	Overcoming Resistance by Using
	Action 607		Combinations of Drugs 640
25.4.1	PG Assembly Is Targeted by Diverse	26.9	Conclusions 641
	Antibiotics 607		Questions 641
25.4.2	Penicillin and Other Antibiotics Induce		References 642
	Dominant-Negative Effects 609		
25.4.3	Identifying Inhibitors of Essential Enzymes Is	27	Chemical Developmental
	Not Enough 609		Biology 647
25.4.4	Identifying Attributes for Compound Uptake		James K. Chen
	in Bacteria 610	27.1	Introduction 647
25.5	Chemical Biology Strategies for Imaging PG	27.2	Small-Molecule Teratogens 648
	Assembly and Remodeling 610	27.2.1	Cyclopamine 648
25.5.1	Antibiotic-Based PG Probes 611	27.2.2	Thalidomide 650
	Antibiotic-based (G (100es 011	21.2.2	i iiaiidoiiiide 050
	Antibiotics that Bind PG Intermediates 611		
		27.2.2 27.3 27.3.1	Optochemical and Optogenetic Probes 653 Optochemical Control of Gene

	C
(iv	Content

27.3.2	Optogenetic Control of Cell Signaling 657	29.3	Visualization 708
27.4	Lineage Tracing Tools 660	29.3.1	Chemical Staining and Imaging Methods
27.4.1	Chemical Control of Genetic		Have Launched Modern Neuroscience 708
	Recombination 661	29.3.2	Calcium Imaging Can Be Used to Monitor
27.4.2	DNA Barcoding Strategies 664		Neuronal Activity 708
27.5	Summary 665	29.3.3	Voltage Sensing Provides a Direct Picture of
	Questions 665		Neuronal Activity 708
	References 665	29.3.4	Neurotransmitters Can Be Sensed with
			Chemogenetic FRET Sensors 708
28	Chemical Immunology 669	29.3.5	Metals and Gases in the Brain Can Be Sensed
	Matthew E. Griffin, John Teijaro, and		with Fluorescent Probes 709
	Howard C. Hang	29.3.6	Positron Emission Tomography Requires Fast
28.1	Introduction 669		Chemistry 712
28.2	Chemical Dissection of Adaptive	29.3.7	Proximity Ligation Enables Spatially Resolved
	Immunity 669		Mapping of Neural Networks 713
28.3	Generation and Chemical Engineering of	29.4	Summary and Outlook 715
	Antibodies 672		Questions 715
28.4	Antigen Recognition by Immune		References 715
	Cells 673		
28.5	Chemical Innovations for Eliciting and	30	Small-Molecule Drug
	Discovering Antigen-specific Immune		Discovery 723
	Responses 676		Luke L. Lairson
28.6	Chemical Modulation of Innate	30.1	Introduction 723
	Immunity 678	30.2	Discovery of Chemical Matter 724
28.7	Chemical Dissection of Immunity 682	30.2.1	Target-Based Discovery 724
28.8	Summary and Future Outlook 685	30.2.2	HTS-Compatible Assay Formats 725
	Questions 685	30.2.3	Phenotype-Based Discovery 727
	References 685	30.2.4	HTS: General Considerations 728
		30.2.5	Drug Repurposing and Serendipity 729
29	Chemical Neurobiology 695	30.2.6	Alternative Small-Molecule Discovery
	Johannes Morstein and Dirk Trauner		Approaches 729
29.1	Introduction 695	30.3	In Vivo Pharmacology: Invention of Drug
29.2	Actuation 697		Candidates and In Vivo Probes 730
29.2.1	Neuropharmacology Has a Storied	30.3.1	Drug Absorption, Distribution, Metabolism,
	History 697		and Excretion 731
29.2.2	Molecular Cloning and Structural Biology	30.3.1.1	Drug Absorption and Distribution 731
	Have Revolutionized the Field 697	30.3.1.2	Physicochemical Properties of Drugs 733
29.2.3	Caged Ligands and Photopharmacology		Drug Metabolism and Excretion 734
	Allow for Optical Control of Neural		Pharmacokinetics, Pharmacodynamics, and
	Activity 699		Biomarkers 737
29.2.4	Chemogenetics Enables Cell-Specific	30.3.2	Medicinal Chemistry 739
	Neuropharmacology in Brains 701	30.3.3	Drug Toxicity and Human Clinical
29.2.5	Tethered Pharmacology Operates on		Trials 743
	Engineered Receptors or Native Receptors in	30.4	Conclusion 744
	Genetically Modified Cells 703		Questions 744
29.2.6	Tethered Photopharmacology Combines		References 746
	Genetic with Optical Control 704		
29.2.7	Synthetic Photoreceptors Can Be Engineered		Index 751
	Through Genetic Code Expansion 705		