## **Contents**

List of Contributors XVAbout the Series Editors XXIII

1	Integrative Analysis of Omics Data 1 Tobias Österlund, Marija Cvijovic, and Erik Kristiansson
	Summary 1
1.1	Introduction 1
1.2	Omics Data and Their Measurement Platforms 4
1.2.1	Omics Data Types 4
1.2.2	Measurement Platforms 5
1.3	Data Processing: Quality Assessment, Quantification, Normalization
1.5	and Statistical Analysis 6
1.3.1	Quality Assessment 7
1.3.2	Quantification 9
1.3.3	Normalization 10
1.3.4	Statistical Analysis 11
1.4	Data Integration: From a List of Genes to Biological Meaning 12
1.4.1	Data Resources for Constructing Gene Sets 13
1.4.1.1	Gene Ontology Terms 13
1.4.1.2	KEGG and Reactome 13
1.4.1.3	Genome-Scale Metabolic Reconstructions 14
1.4.2	Gene Set Analysis 14
1.4.2.1	Gene Set Overenrichment Tests 16
1.4.2.2	Rank-Based Enrichment Tests 16
1.4.3	Networks and Network Topology 17
1.5	Outlook and Perspectives 18
	References 19
2	<sup>13</sup> C Flux Analysis in Biotechnology and Medicine 25
	Yi Ern Cheah, Clinton M. Hasenour, and Jamey D. Young
2.1	Introduction 25
2.1.1	Why Study Metabolic Fluxes? 25
2.1.2	Why are Isotope Tracers Important for Flux Analysis? 26

2.1.3 How are Fluxes Determined? 28 2.2 Theoretical Foundations of <sup>13</sup> C MFA 29 2.2.1 Elementary Metabolite Units (EMUs) 30 2.2.2 Flux Uncertainty Analysis 31 2.2.3 Optimal Design of Isotope Labeling Experiments 32 2.2.4 Isotopically Nonstationary MFA (INST-MFA) 34 2.3 Metabolic Flux Analysis in Biotechnology 36 2.3.1 <sup>13</sup> C MFA for Host Characterization 36 2.3.2 <sup>13</sup> C MFA for Bottleneck Identification 41 2.4 Metabolic Flux Analysis in Medicine 42 2.4.1 Liver Glucose and Oxidative Metabolism 43 2.4.2 Cancer Cell Metabolism 47 2.4.3 Fuel Oxidation and Anaplerosis in the Heart 48 2.4.4 Metabolism in Other Tissues: Pancreas, Brain, Muscle, Adipose, and Immune Cells 49 2.5 Emerging Challenges for <sup>13</sup> C MFA 50 2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50 2.5.2 Genome-Scale <sup>13</sup> C MFA 51 2.5.3 New Measurement Strategies 52 2.5.4 High-Throughput MFA 53 2.5.5 Application of MFA to Industrial Bioprocesses 53 1.1 Integrating MFA with Omics Measurements 54 2.6 Conclusion 55 Acknowledgments 55 Disclosure 55 References 55 3 Metabolic Modeling for Design of Cell Factories 71 Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed Summary 71 3.1 Introduction 71 3.2 Building and Refining Genome-Scale Metabolic Models 72 3.2.1 Generate a Draft Metabolic Network (Step 1) 74 3.2.2 Manually Curate the Draft Metabolic Network (Step 1) 74 3.2.3 Develop a Constraint-Based Model (Step 3) 77 3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79 3.2.5 Predicting the Effects of Genetic Manipulations 81 3.3 Strain Design Algorithms 83 3.3.1 Fundamentals of Bilevel Optimization 84 3.3.2 Algorithms Involving Gene Additions 94 3.3.3 Algorithms Involving Gene Pover/Underexpression 95	Contents	
2.2 Theoretical Foundations of <sup>13</sup> C MFA 29 2.2.1 Elementary Metabolite Units (EMUs) 30 2.2.2 Flux Uncertainty Analysis 31 2.2.3 Optimal Design of Isotope Labeling Experiments 32 2.2.4 Isotopically Nonstationary MFA (INST-MFA) 34 2.3 Metabolic Flux Analysis in Biotechnology 36 2.3.1 <sup>13</sup> C MFA for Host Characterization 36 2.3.2 <sup>13</sup> C MFA for Pinpointing Yield Losses and Futile Cycles 39 2.3.3 <sup>13</sup> C MFA for Bottleneck Identification 41 2.4 Metabolic Flux Analysis in Medicine 42 2.4.1 Liver Glucose and Oxidative Metabolism 43 2.4.2 Cancer Cell Metabolism 47 2.4.3 Fuel Oxidation and Anaplerosis in the Heart 48 2.4.4 Metabolism in Other Tissues: Pancreas, Brain, Muscle, Adipose, and Immune Cells 49 2.5 Emerging Challenges for <sup>13</sup> C MFA 50 2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50 2.5.2 Genome-Scale <sup>13</sup> C MFA 51 2.5.3 New Measurement Strategies 52 2.5.4 High-Throughput MFA 53 2.5.5 Application of MFA to Industrial Bioprocesses 53 2.5.6 Integrating MFA with Omics Measurements 54 2.6 Conclusion 55 Acknowledgments 55 Disclosure 55 References 55 3 Metabolic Modeling for Design of Cell Factories 71 Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed Summary 71 3.1 Introduction 71 3.2 Building and Refining Genome-Scale Metabolic Models 72 3.2.1 Generate a Draft Metabolic Network (Step 1) 74 3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75 3.2.3 Develop a Constraint-Based Model (Step 3) 77 3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79 3.2.5 Predicting the Effects of Genetic Manipulations 81 3.3 Strain Design Algorithms 83 3.3.1 Fundamentals of Bilevel Optimization 84 3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94 3.3.3 Algorithms Involving Gene Additions 94	2.1.3	How are Fluxes Determined? 28
2.2.1 Elementary Metabolite Units (EMUs) 30 2.2.2 Flux Uncertainty Analysis 31 2.2.3 Optimal Design of Isotope Labeling Experiments 32 2.2.4 Isotopically Nonstationary MFA (INST-MFA) 34 2.3 Metabolic Flux Analysis in Biotechnology 36 2.3.1 13°C MFA for Host Characterization 36 2.3.2 13°C MFA for Pinpointing Yield Losses and Futile Cycles 39 2.3.3 13°C MFA for Bottleneck Identification 41 2.4 Metabolic Flux Analysis in Medicine 42 2.4.1 Liver Glucose and Oxidative Metabolism 43 2.4.2 Cancer Cell Metabolism 47 2.4.3 Fuel Oxidation and Anaplerosis in the Heart 48 2.4.4 Metabolism in Other Tissues: Pancreas, Brain, Muscle, Adipose, and Immune Cells 49 2.5 Emerging Challenges for 13°C MFA 50 2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50 2.5.2 Genome-Scale 13°C MFA 51 2.5.3 New Measurement Strategies 52 2.5.4 High-Throughput MFA 53 2.5.5 Application of MFA to Industrial Bioprocesses 53 2.5.6 Integrating MFA with Omics Measurements 54 2.6 Conclusion 55 Acknowledgments 55 Disclosure 55 References 55 3 Metabolic Modeling for Design of Cell Factories 71 Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed Summary 71 3.1 Introduction 71 3.2 Building and Refining Genome-Scale Metabolic Models 72 3.2.1 Generate a Draft Metabolic Network (Step 1) 74 3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75 3.2.3 Develop a Constraint-Based Model (Step 3) 77 3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79 3.2.5 Predicting the Effects of Genetic Manipulations 81 3.3 Strain Design Algorithms 83 3.3.1 Fundamentals of Bilevel Optimization 84 3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94 3.3.3 Algorithms Involving Gene Additions 94	2.2	Theoretical Foundations of <sup>13</sup> C MFA 29
2.2.2 Flux Uncertainty Analysis 31 2.2.3 Optimal Design of Isotope Labeling Experiments 32 2.2.4 Isotopically Nonstationary MFA (INST-MFA) 34 2.3 Metabolic Flux Analysis in Biotechnology 36 2.3.1 13°C MFA for Host Characterization 36 2.3.2 13°C MFA for Pinpointing Yield Losses and Futile Cycles 39 2.3.3 13°C MFA for Bottleneck Identification 41 2.4 Metabolic Flux Analysis in Medicine 42 2.4.1 Liver Glucose and Oxidative Metabolism 43 2.4.2 Cancer Cell Metabolism 47 2.4.3 Fuel Oxidation and Anaplerosis in the Heart 48 2.4.4 Metabolism in Other Tissues: Pancreas, Brain, Muscle, Adipose, and Immune Cells 49 2.5 Emerging Challenges for 13°C MFA 50 2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50 2.5.2 Genome-Scale 13°C MFA 51 2.5.3 New Measurement Strategies 52 2.5.4 High-Throughput MFA 53 2.5.5 Application of MFA to Industrial Bioprocesses 53 1.1 Integrating MFA with Omics Measurements 54 2.6 Conclusion 55 Acknowledgments 55 Disclosure 55 References 55 3 Metabolic Modeling for Design of Cell Factories 71 Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed Summary 71 3.1 Introduction 71 3.2 Building and Refining Genome-Scale Metabolic Models 72 3.2.1 Generate a Draft Metabolic Network (Step 1) 74 3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75 3.2.3 Develop a Constraint-Based Model (Step 3) 77 3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79 3.2.5 Predicting the Effects of Genetic Manipulations 81 3.3 Strain Design Algorithms 83 3.3.1 Fundamentals of Bilevel Optimization 84 3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94 3.3.3 Algorithms Involving Gene Additions 94	2.2.1	
2.2.3 Optimal Design of Isotope Labeling Experiments 32 2.2.4 Isotopically Nonstationary MFA (INST-MFA) 34 2.3 Metabolic Flux Analysis in Biotechnology 36 2.3.1 13C MFA for Host Characterization 36 2.3.2 13C MFA for Pinpointing Yield Losses and Futile Cycles 39 2.3.3 13C MFA for Bottleneck Identification 41 2.4 Metabolic Flux Analysis in Medicine 42 2.4.1 Liver Glucose and Oxidative Metabolism 43 2.4.2 Cancer Cell Metabolism 47 2.4.3 Fuel Oxidation and Anaplerosis in the Heart 48 2.4.4 Metabolism in Other Tissues: Pancreas, Brain, Muscle, Adipose, and Immune Cells 49 2.5 Emerging Challenges for 13C MFA 50 2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50 2.5.2 Genome-Scale 13C MFA 51 2.5.3 New Measurement Strategies 52 2.5.4 High-Throughput MFA 53 2.5.5 Application of MFA to Industrial Bioprocesses 53 2.5.6 Integrating MFA with Omics Measurements 54 2.6 Conclusion 55 Acknowledgments 55 Disclosure 55 References 55 3 Metabolic Modeling for Design of Cell Factories 71 Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed Summary 71 3.1 Introduction 71 3.2 Building and Refining Genome-Scale Metabolic Models 72 3.2.1 Generate a Draft Metabolic Network (Step 1) 74 3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75 3.2.3 Develop a Constraint-Based Model (Step 3) 77 3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79 3.2.5 Predicting the Effects of Genetic Manipulations 81 3.3 Strain Design Algorithms 83 3.3.1 Fundamentals of Bilevel Optimization 84 3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94 3.3.3 Algorithms Involving Gene Additions 94	2.2.2	·
2.2.4 Isotopically Nonstationary MFA (INST-MFA) 34 2.3 Metabolic Flux Analysis in Biotechnology 36 2.3.1 13C MFA for Host Characterization 36 2.3.2 13C MFA for Pinpointing Yield Losses and Futile Cycles 39 2.3.3 13C MFA for Bottleneck Identification 41 2.4 Metabolic Flux Analysis in Medicine 42 2.4.1 Liver Glucose and Oxidative Metabolism 43 2.4.2 Cancer Cell Metabolism 47 2.4.3 Fuel Oxidation and Anaplerosis in the Heart 48 2.4.4 Metabolism in Other Tissues: Pancreas, Brain, Muscle, Adipose, and Immune Cells 49 2.5 Emerging Challenges for 13C MFA 50 2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50 2.5.2 Genome-Scale 13C MFA 51 2.5.3 New Measurement Strategies 52 2.5.4 High-Throughput MFA 53 2.5.5 Application of MFA to Industrial Bioprocesses 53 2.5.6 Integrating MFA with Omics Measurements 54 2.6 Conclusion 55 Acknowledgments 55 Disclosure 55 References 55  3 Metabolic Modeling for Design of Cell Factories 71 Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed Summary 71 3.1 Introduction 71 3.2 Building and Refining Genome-Scale Metabolic Models 72 3.2.1 Generate a Draft Metabolic Network (Step 1) 74 3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75 3.2.3 Develop a Constraint-Based Model (Step 3) 77 3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79 3.2.5 Predicting the Effects of Genetic Manipulations 81 3.3 Strain Design Algorithms 83 3.3.1 Fundamentals of Bilevel Optimization 84 3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94 3.3.3 Algorithms Involving Gene Additions 94	2.2.3	·
<ul> <li>2.3.1 13C MFA for Host Characterization 36</li> <li>2.3.2 13C MFA for Pinpointing Yield Losses and Futile Cycles 39</li> <li>2.3.3 13C MFA for Bottleneck Identification 41</li> <li>2.4 Metabolic Flux Analysis in Medicine 42</li> <li>2.4.1 Liver Glucose and Oxidative Metabolism 43</li> <li>2.4.2 Cancer Cell Metabolism 47</li> <li>2.4.3 Fuel Oxidation and Anaplerosis in the Heart 48</li> <li>2.4.4 Metabolism in Other Tissues: Pancreas, Brain, Muscle, Adipose, and Immune Cells 49</li> <li>2.5 Emerging Challenges for 13C MFA 50</li> <li>2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50</li> <li>2.5.2 Genome-Scale 13C MFA 51</li> <li>2.5.3 New Measurement Strategies 52</li> <li>2.5.4 High-Throughput MFA 53</li> <li>2.5.5 Application of MFA to Industrial Bioprocesses 53</li> <li>2.5.6 Integrating MFA with Omics Measurements 54</li> <li>2.6 Conclusion 55</li></ul>	2.2.4	
<ul> <li>2.3.1 13C MFA for Host Characterization 36</li> <li>2.3.2 13C MFA for Pinpointing Yield Losses and Futile Cycles 39</li> <li>2.3.3 13C MFA for Bottleneck Identification 41</li> <li>2.4 Metabolic Flux Analysis in Medicine 42</li> <li>2.4.1 Liver Glucose and Oxidative Metabolism 43</li> <li>2.4.2 Cancer Cell Metabolism 47</li> <li>2.4.3 Fuel Oxidation and Anaplerosis in the Heart 48</li> <li>2.4.4 Metabolism in Other Tissues: Pancreas, Brain, Muscle, Adipose, and Immune Cells 49</li> <li>2.5 Emerging Challenges for 13C MFA 50</li> <li>2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50</li> <li>2.5.2 Genome-Scale 13C MFA 51</li> <li>2.5.3 New Measurement Strategies 52</li> <li>2.5.4 High-Throughput MFA 53</li> <li>2.5.5 Application of MFA to Industrial Bioprocesses 53</li> <li>2.5.6 Integrating MFA with Omics Measurements 54</li> <li>2.6 Conclusion 55</li></ul>	2.3	Metabolic Flux Analysis in Biotechnology 36
<ul> <li>2.3.3 13C MFA for Bottleneck Identification 41</li> <li>2.4 Metabolic Flux Analysis in Medicine 42</li> <li>2.4.1 Liver Glucose and Oxidative Metabolism 43</li> <li>2.4.2 Cancer Cell Metabolism 47</li> <li>2.4.3 Fuel Oxidation and Anaplerosis in the Heart 48</li> <li>2.4.4 Metabolism in Other Tissues: Pancreas, Brain, Muscle, Adipose, and Immune Cells 49</li> <li>2.5 Emerging Challenges for 13C MFA 50</li> <li>2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50</li> <li>2.5.2 Genome-Scale 13C MFA 51</li> <li>2.5.3 New Measurement Strategies 52</li> <li>2.5.4 High-Throughput MFA 53</li> <li>2.5.5 Application of MFA to Industrial Bioprocesses 53</li> <li>2.5.6 Integrating MFA with Omics Measurements 54</li> <li>2.6 Conclusion 55</li></ul>	2.3.1	<sup>13</sup> C MFA for Host Characterization 36
<ul> <li>2.3.3 13C MFA for Bottleneck Identification 41</li> <li>2.4 Metabolic Flux Analysis in Medicine 42</li> <li>2.4.1 Liver Glucose and Oxidative Metabolism 43</li> <li>2.4.2 Cancer Cell Metabolism 47</li> <li>2.4.3 Fuel Oxidation and Anaplerosis in the Heart 48</li> <li>2.4.4 Metabolism in Other Tissues: Pancreas, Brain, Muscle, Adipose, and Immune Cells 49</li> <li>2.5 Emerging Challenges for 13C MFA 50</li> <li>2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50</li> <li>2.5.2 Genome-Scale 13C MFA 51</li> <li>2.5.3 New Measurement Strategies 52</li> <li>2.5.4 High-Throughput MFA 53</li> <li>2.5.5 Application of MFA to Industrial Bioprocesses 53</li> <li>2.5.6 Integrating MFA with Omics Measurements 54</li> <li>2.6 Conclusion 55</li></ul>	2.3.2	<sup>13</sup> C MFA for Pinpointing Yield Losses and Futile Cycles 39
<ul> <li>2.4.1 Liver Glucose and Oxidative Metabolism 43</li> <li>2.4.2 Cancer Cell Metabolism 47</li> <li>2.4.3 Fuel Oxidation and Anaplerosis in the Heart 48</li> <li>2.4.4 Metabolism in Other Tissues: Pancreas, Brain, Muscle, Adipose, and Immune Cells 49</li> <li>2.5 Emerging Challenges for <sup>13</sup>C MFA 50</li> <li>2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50</li> <li>2.5.2 Genome-Scale <sup>13</sup>C MFA 51</li> <li>2.5.3 New Measurement Strategies 52</li> <li>2.5.4 High-Throughput MFA 53</li> <li>2.5.5 Application of MFA to Industrial Bioprocesses 53</li> <li>2.5.6 Integrating MFA with Omics Measurements 54</li> <li>2.6 Conclusion 55</li></ul>	2.3.3	
<ul> <li>2.4.2 Cancer Cell Metabolism 47</li> <li>2.4.3 Fuel Oxidation and Anaplerosis in the Heart 48</li> <li>2.4.4 Metabolism in Other Tissues: Pancreas, Brain, Muscle, Adipose, and Immune Cells 49</li> <li>2.5 Emerging Challenges for <sup>13</sup>C MFA 50</li> <li>2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50</li> <li>2.5.2 Genome-Scale <sup>13</sup>C MFA 51</li> <li>2.5.3 New Measurement Strategies 52</li> <li>2.5.4 High-Throughput MFA 53</li> <li>2.5.5 Application of MFA to Industrial Bioprocesses 53</li> <li>2.5.6 Integrating MFA with Omics Measurements 54</li> <li>2.6 Conclusion 55</li></ul>	2.4	Metabolic Flux Analysis in Medicine 42
<ul> <li>2.4.3 Fuel Oxidation and Anaplerosis in the Heart 48</li> <li>2.4.4 Metabolism in Other Tissues: Pancreas, Brain, Muscle, Adipose, and Immune Cells 49</li> <li>2.5 Emerging Challenges for <sup>13</sup>C MFA 50</li> <li>2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50</li> <li>2.5.2 Genome-Scale <sup>13</sup>C MFA 51</li> <li>2.5.3 New Measurement Strategies 52</li> <li>2.5.4 High-Throughput MFA 53</li> <li>2.5.5 Application of MFA to Industrial Bioprocesses 53</li> <li>2.5.6 Integrating MFA with Omics Measurements 54</li> <li>2.6 Conclusion 55</li></ul>	2.4.1	Liver Glucose and Oxidative Metabolism 43
<ul> <li>2.4.4 Metabolism in Other Tissues: Pancreas, Brain, Muscle, Adipose, and Immune Cells 49</li> <li>2.5 Emerging Challenges for <sup>13</sup>C MFA 50</li> <li>2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50</li> <li>2.5.2 Genome-Scale <sup>13</sup>C MFA 51</li> <li>2.5.3 New Measurement Strategies 52</li> <li>2.5.4 High-Throughput MFA 53</li> <li>2.5.5 Application of MFA to Industrial Bioprocesses 53</li> <li>2.5.6 Integrating MFA with Omics Measurements 54</li> <li>2.6 Conclusion 55</li></ul>	2.4.2	Cancer Cell Metabolism 47
Immune Cells 49  2.5 Emerging Challenges for <sup>13</sup> C MFA 50  2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50  2.5.2 Genome-Scale <sup>13</sup> C MFA 51  2.5.3 New Measurement Strategies 52  2.5.4 High-Throughput MFA 53  2.5.5 Application of MFA to Industrial Bioprocesses 53  2.5.6 Integrating MFA with Omics Measurements 54  2.6 Conclusion 55     Acknowledgments 55     Disclosure 55     References 55  3 Metabolic Modeling for Design of Cell Factories 71     Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed Summary 71  3.1 Introduction 71  3.2 Building and Refining Genome-Scale Metabolic Models 72  3.2.1 Generate a Draft Metabolic Network (Step 1) 74  3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75  3.2.3 Develop a Constraint-Based Model (Step 3) 77  3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79  3.2.5 Predicting the Effects of Genetic Manipulations 81  3.3 Strain Design Algorithms 83  3.3.1 Fundamentals of Bilevel Optimization 84  3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94  3.3.3 Algorithms Involving Gene Additions 94	2.4.3	Fuel Oxidation and Anaplerosis in the Heart 48
<ul> <li>2.5 Emerging Challenges for <sup>13</sup>C MFA 50</li> <li>2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50</li> <li>2.5.2 Genome-Scale <sup>13</sup>C MFA 51</li> <li>2.5.3 New Measurement Strategies 52</li> <li>2.5.4 High-Throughput MFA 53</li> <li>2.5.5 Application of MFA to Industrial Bioprocesses 53</li> <li>2.5.6 Integrating MFA with Omics Measurements 54</li> <li>2.6 Conclusion 55</li></ul>	2.4.4	Metabolism in Other Tissues: Pancreas, Brain, Muscle, Adipose, and
2.5.1 Theoretical and Computational Advances: Multiple Tracers, Co-culture MFA, Dynamic MFA 50  2.5.2 Genome-Scale <sup>13</sup> C MFA 51  2.5.3 New Measurement Strategies 52  2.5.4 High-Throughput MFA 53  2.5.5 Application of MFA to Industrial Bioprocesses 53  2.5.6 Integrating MFA with Omics Measurements 54  2.6 Conclusion 55     Acknowledgments 55     Disclosure 55     References 55  3 Metabolic Modeling for Design of Cell Factories 71     Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed     Summary 71  3.1 Introduction 71  3.2 Building and Refining Genome-Scale Metabolic Models 72  3.2.1 Generate a Draft Metabolic Network (Step 1) 74  3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75  3.2.3 Develop a Constraint-Based Model (Step 3) 77  3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79  3.2.5 Predicting the Effects of Genetic Manipulations 81  3.3 Strain Design Algorithms 83  3.3.1 Fundamentals of Bilevel Optimization 84  3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94  3.3.3 Algorithms Involving Gene Additions 94		Immune Cells 49
Co-culture MFA, Dynamic MFA 50  2.5.2 Genome-Scale <sup>13</sup> C MFA 51  2.5.3 New Measurement Strategies 52  2.5.4 High-Throughput MFA 53  2.5.5 Application of MFA to Industrial Bioprocesses 53  2.5.6 Integrating MFA with Omics Measurements 54  2.6 Conclusion 55     Acknowledgments 55     Disclosure 55     References 55  3 Metabolic Modeling for Design of Cell Factories 71     Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed Summary 71  3.1 Introduction 71  3.2 Building and Refining Genome-Scale Metabolic Models 72  3.2.1 Generate a Draft Metabolic Network (Step 1) 74  3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75  3.2.3 Develop a Constraint-Based Model (Step 3) 77  3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79  3.2.5 Predicting the Effects of Genetic Manipulations 81  3.3 Strain Design Algorithms 83  3.3.1 Fundamentals of Bilevel Optimization 84  3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94  3.3.3 Algorithms Involving Gene Additions 94	2.5	Emerging Challenges for <sup>13</sup> C MFA 50
<ul> <li>2.5.2 Genome-Scale <sup>13</sup>C MFA 51</li> <li>2.5.3 New Measurement Strategies 52</li> <li>2.5.4 High-Throughput MFA 53</li> <li>2.5.5 Application of MFA to Industrial Bioprocesses 53</li> <li>2.5.6 Integrating MFA with Omics Measurements 54</li> <li>2.6 Conclusion 55</li></ul>	2.5.1	Theoretical and Computational Advances: Multiple Tracers,
<ul> <li>2.5.3 New Measurement Strategies 52</li> <li>2.5.4 High-Throughput MFA 53</li> <li>2.5.5 Application of MFA to Industrial Bioprocesses 53</li> <li>2.5.6 Integrating MFA with Omics Measurements 54</li> <li>2.6 Conclusion 55</li></ul>		Co-culture MFA, Dynamic MFA 50
<ul> <li>2.5.4 High-Throughput MFA 53</li> <li>2.5.5 Application of MFA to Industrial Bioprocesses 53</li> <li>2.5.6 Integrating MFA with Omics Measurements 54</li> <li>2.6 Conclusion 55</li></ul>	2.5.2	Genome-Scale <sup>13</sup> C MFA 51
<ul> <li>2.5.5 Application of MFA to Industrial Bioprocesses 53</li> <li>2.5.6 Integrating MFA with Omics Measurements 54</li> <li>2.6 Conclusion 55</li></ul>	2.5.3	New Measurement Strategies 52
<ul> <li>2.5.6 Integrating MFA with Omics Measurements 54</li> <li>2.6 Conclusion 55</li></ul>	2.5.4	High-Throughput MFA 53
2.6 Conclusion 55 Acknowledgments 55 Disclosure 55 References 55  3 Metabolic Modeling for Design of Cell Factories 71 Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed Summary 71 3.1 Introduction 71 3.2 Building and Refining Genome-Scale Metabolic Models 72 3.2.1 Generate a Draft Metabolic Network (Step 1) 74 3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75 3.2.3 Develop a Constraint-Based Model (Step 3) 77 3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79 3.2.5 Predicting the Effects of Genetic Manipulations 81 3.3 Strain Design Algorithms 83 3.3.1 Fundamentals of Bilevel Optimization 84 3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94 3.3.3 Algorithms Involving Gene Additions 94	2.5.5	Application of MFA to Industrial Bioprocesses 53
Acknowledgments 55 Disclosure 55 References 55  Metabolic Modeling for Design of Cell Factories 71 Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed Summary 71  3.1 Introduction 71  3.2 Building and Refining Genome-Scale Metabolic Models 72  3.2.1 Generate a Draft Metabolic Network (Step 1) 74  3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75  3.2.3 Develop a Constraint-Based Model (Step 3) 77  3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79  3.2.5 Predicting the Effects of Genetic Manipulations 81  3.3 Strain Design Algorithms 83  3.3.1 Fundamentals of Bilevel Optimization 84  3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94  3.3.3 Algorithms Involving Gene Additions 94	2.5.6	Integrating MFA with Omics Measurements 54
Disclosure 55 References 55  References 55  References 55  Metabolic Modeling for Design of Cell Factories 71 Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed Summary 71  3.1 Introduction 71  3.2 Building and Refining Genome-Scale Metabolic Models 72  3.2.1 Generate a Draft Metabolic Network (Step 1) 74  3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75  3.2.3 Develop a Constraint-Based Model (Step 3) 77  3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79  3.2.5 Predicting the Effects of Genetic Manipulations 81  3.3 Strain Design Algorithms 83  3.3.1 Fundamentals of Bilevel Optimization 84  3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94  3.3.3 Algorithms Involving Gene Additions 94	2.6	Conclusion 55
References 55  Metabolic Modeling for Design of Cell Factories 71 Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed Summary 71  3.1 Introduction 71  3.2 Building and Refining Genome-Scale Metabolic Models 72  3.2.1 Generate a Draft Metabolic Network (Step 1) 74  3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75  3.2.3 Develop a Constraint-Based Model (Step 3) 77  3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79  3.2.5 Predicting the Effects of Genetic Manipulations 81  3.3 Strain Design Algorithms 83  3.3.1 Fundamentals of Bilevel Optimization 84  3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94  3.3.3 Algorithms Involving Gene Additions 94		Acknowledgments 55
Metabolic Modeling for Design of Cell Factories 71 Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed Summary 71  3.1 Introduction 71  3.2 Building and Refining Genome-Scale Metabolic Models 72  3.2.1 Generate a Draft Metabolic Network (Step 1) 74  3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75  3.2.3 Develop a Constraint-Based Model (Step 3) 77  3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79  3.2.5 Predicting the Effects of Genetic Manipulations 81  3.3 Strain Design Algorithms 83  3.3.1 Fundamentals of Bilevel Optimization 84  3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94  3.3.3 Algorithms Involving Gene Additions 94		Disclosure 55
Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed Summary 71  3.1 Introduction 71  3.2 Building and Refining Genome-Scale Metabolic Models 72  3.2.1 Generate a Draft Metabolic Network (Step 1) 74  3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75  3.2.3 Develop a Constraint-Based Model (Step 3) 77  3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79  3.2.5 Predicting the Effects of Genetic Manipulations 81  3.3 Strain Design Algorithms 83  3.3.1 Fundamentals of Bilevel Optimization 84  3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94  3.3.3 Algorithms Involving Gene Additions 94		References 55
Mingyuan Tian, Prashant Kumar, Sanjan T. P. Gupta, and Jennifer L. Reed Summary 71  3.1 Introduction 71  3.2 Building and Refining Genome-Scale Metabolic Models 72  3.2.1 Generate a Draft Metabolic Network (Step 1) 74  3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75  3.2.3 Develop a Constraint-Based Model (Step 3) 77  3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79  3.2.5 Predicting the Effects of Genetic Manipulations 81  3.3 Strain Design Algorithms 83  3.3.1 Fundamentals of Bilevel Optimization 84  3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94  3.3.3 Algorithms Involving Gene Additions 94	3	Metabolic Modeling for Design of Cell Factories 71
Summary 71 3.1 Introduction 71 3.2 Building and Refining Genome-Scale Metabolic Models 72 3.2.1 Generate a Draft Metabolic Network (Step 1) 74 3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75 3.2.3 Develop a Constraint-Based Model (Step 3) 77 3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79 3.2.5 Predicting the Effects of Genetic Manipulations 81 3.3 Strain Design Algorithms 83 3.3.1 Fundamentals of Bilevel Optimization 84 3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94 3.3.3 Algorithms Involving Gene Additions 94	•	
3.1 Introduction 71 3.2 Building and Refining Genome-Scale Metabolic Models 72 3.2.1 Generate a Draft Metabolic Network (Step 1) 74 3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75 3.2.3 Develop a Constraint-Based Model (Step 3) 77 3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79 3.2.5 Predicting the Effects of Genetic Manipulations 81 3.3 Strain Design Algorithms 83 3.3.1 Fundamentals of Bilevel Optimization 84 3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94 3.3.3 Algorithms Involving Gene Additions 94		
3.2 Building and Refining Genome-Scale Metabolic Models 72 3.2.1 Generate a Draft Metabolic Network (Step 1) 74 3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75 3.2.3 Develop a Constraint-Based Model (Step 3) 77 3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79 3.2.5 Predicting the Effects of Genetic Manipulations 81 3.3 Strain Design Algorithms 83 3.3.1 Fundamentals of Bilevel Optimization 84 3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94 3.3.3 Algorithms Involving Gene Additions 94	3.1	· · · · · · · · · · · · · · · · · · ·
<ul> <li>3.2.1 Generate a Draft Metabolic Network (Step 1) 74</li> <li>3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75</li> <li>3.2.3 Develop a Constraint-Based Model (Step 3) 77</li> <li>3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79</li> <li>3.2.5 Predicting the Effects of Genetic Manipulations 81</li> <li>3.3 Strain Design Algorithms 83</li> <li>3.3.1 Fundamentals of Bilevel Optimization 84</li> <li>3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94</li> <li>3.3.3 Algorithms Involving Gene Additions 94</li> </ul>		
<ul> <li>3.2.2 Manually Curate the Draft Metabolic Network (Step 2) 75</li> <li>3.2.3 Develop a Constraint-Based Model (Step 3) 77</li> <li>3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79</li> <li>3.2.5 Predicting the Effects of Genetic Manipulations 81</li> <li>3.3 Strain Design Algorithms 83</li> <li>3.3.1 Fundamentals of Bilevel Optimization 84</li> <li>3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94</li> <li>3.3.3 Algorithms Involving Gene Additions 94</li> </ul>		
3.2.3 Develop a Constraint-Based Model (Step 3) 77 3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79 3.2.5 Predicting the Effects of Genetic Manipulations 81 3.3 Strain Design Algorithms 83 3.3.1 Fundamentals of Bilevel Optimization 84 3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94 3.3.3 Algorithms Involving Gene Additions 94		
3.2.4 Revise the Metabolic Model through Reconciliation with Experimental Data (Step 4) 79 3.2.5 Predicting the Effects of Genetic Manipulations 81 3.3 Strain Design Algorithms 83 3.3.1 Fundamentals of Bilevel Optimization 84 3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94 3.3.3 Algorithms Involving Gene Additions 94	3.2.3	
Experimental Data (Step 4) 79  3.2.5 Predicting the Effects of Genetic Manipulations 81  3.3 Strain Design Algorithms 83  3.3.1 Fundamentals of Bilevel Optimization 84  3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94  3.3.3 Algorithms Involving Gene Additions 94		•
<ul> <li>3.2.5 Predicting the Effects of Genetic Manipulations 81</li> <li>3.3 Strain Design Algorithms 83</li> <li>3.3.1 Fundamentals of Bilevel Optimization 84</li> <li>3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94</li> <li>3.3.3 Algorithms Involving Gene Additions 94</li> </ul>		
3.3 Strain Design Algorithms 83 3.3.1 Fundamentals of Bilevel Optimization 84 3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94 3.3.3 Algorithms Involving Gene Additions 94	3.2.5	•
<ul> <li>3.3.1 Fundamentals of Bilevel Optimization 84</li> <li>3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94</li> <li>3.3.3 Algorithms Involving Gene Additions 94</li> </ul>		
<ul> <li>3.3.2 Algorithms Involving Only Gene/Reaction Deletions 94</li> <li>3.3.3 Algorithms Involving Gene Additions 94</li> </ul>		
3.3.3 Algorithms Involving Gene Additions 94		
· · · · · · · · · · · · · · · · · · ·		- · · · · · · · · · · · · · · · · · · ·

۷I

3.3.5	Algorithms Involving Cofactor Changes 98
3.3.6	Algorithms Involving Multiple Design Criteria 99
3.4	Case Studies 100
3.4.1	Strains Producing Lactate 100
3.4.2	Strains Co-utilizing Sugars 100
3.4.3	Strains Producing 1,4-Butanediol 102
3.5	Conclusions 103
	Acknowledgments 103
	References 104
4	Genome-Scale Metabolic Modeling and <i>In silico</i> Strain Design of
	Escherichia coli 109
	Meiyappan Lakshmanan, Na-Rae Lee, and Dong-Yup Lee
4.1	Introduction 109
4.2	The COBRA Approach 110
4.3	History of <i>E. coli</i> Metabolic Modeling 111
4.3.1	Pre-genomic-era Models 111
4.3.2	Genome-Scale Models 112
4.4	In silico Model-Based Strain Design of E. coli Cell Factories 115
4.4.1	Gene Deletions 127
4.4.2	Gene Up/Downregulations 127
4.4.3	Gene Insertions 128
4.4.4	Cofactor Engineering 128
4.4.5	Other Approaches 128
4.5	Future Directions of Model-Guided Strain Design in E. coli 129
	References 130
5	Accelerating the Drug Development Pipeline with Genome-Scale
	Metabolic Network Reconstructions 139
	Bonnie V. Dougherty, Thomas J. Moutinho Jr., and Jason Papin
	Summary 139
5.1	Introduction 139
5.1.1	Drug Development Pipeline 140
5.1.2	Overview of Genome-Scale Metabolic Network
	Reconstructions 140
5.1.3	Analytical Tools and Mathematical Evaluation 141
5.1.3.1	Flux Balance Analysis (FBA) 141
5.1.3.2	Flux Variability Analysis (FVA) 142
5.2	Metabolic Reconstructions in the Drug Development Pipeline 142
5.2.1	Target Identification 143
5.2.2	Drug Side Effects 145
5.3	Species-Level Microbial Reconstructions 146
5.3.1	Microbial Reconstructions in the Antibiotic Development
	Pipeline 146
5.3.1.1	Applications in the Drug Development Pipeline 146

VIII	Contents	
,	5.3.2	Metabolic-Reconstruction-Facilitated Rational Drug Target Identification 147
	5.3.2.1	Targeting Genes Essential for Biomass Production 147
	5.3.2.2	Targeting Virulence Factors 147
	5.3.2.3	Metabolite-centric Targeting 148
	5.3.3	Repurposing and Expanding Utility of Antibiotics 149
	5.3.3.1	Virtual Drug Screens Informed by Metabolic Reconstructions 149
	5.3.3.2	Limiting Resistance with Drug Combinations 149
	5.3.3.3	Improving Treatment Options by Increasing Sensitivity to Antibiotics 150
	5.3.4	Improving Toxicity Screens with the Human Metabolic Network Reconstruction 150
	5.4	The Human Reconstruction 151
	5.4.1	Approaches for the Human Reconstruction 152
	5.4.2	Target Identification 152
	5.4.2.1	Drug Targeting in Cancer 152
	5.4.2.2	Drug Targeting in Metabolic Diseases 153
	5.4.3	Toxicity and Other Side Effects 154
	5.5	Community Models 155
	5.5.1	Host-Pathogen Community Models 155
	5.5.2	Eukaryotic Community Models 156
	5.6	Personalized Medicine 156
	5.7	Conclusion 157
		References 158
	6	Computational Modeling of Microbial Communities 163 Siu H. J. Chan, Margaret Simons, and Costas D. Maranas Summary 163
	6.1	Introduction 163
	6.1.1	Microbial Communities 163
	6.1.2	Modeling Microbial Communities 165
	6.1.3	Model Structures 165
	6.1.4	Quantitative Approaches 166
	6.2	Ecological Models 168
	6.2.1	Generalized Predator – Prey Model 169
	6.2.2	Evolutionary Game Theory 170
	6.2.3	Models Including Additional Dimensions 171
	6.2.4	Advantages and Disadvantages 171
	6.3	Genome-Scale Metabolic Models 172
	6.3.1	Introduction and Applications 172
	6.3.2	Genome-Scale Metabolic Modeling of Microbial Communities 17-
	6.3.3	Simulation of Microbial Communities Assuming Steady State 175

Predicting Interactions Using FBA 175

Programming 176

Identifying Minimal Media by Mixed Integer Linear

6.3.3.1

6.3.3.2

6.3.3.3	Pareto Optimality Analysis by FBA 176
6.3.3.4	Modeling Chemostat Co-culture 177
6.3.3.5	Community FBA with Community Mass Balance 177
6.3.4	Dynamic Simulation of Multispecies Models 177
6.3.5	Spatial and Temporal Modeling of Communities 178
6.3.6	Using Bilevel Optimization to Capture Multiple Objective
	Functions 179
6.3.6.1	OptCom 179
6.3.6.2	d-OptCom 181
6.3.6.3	CASINO Toolbox 181
6.3.6.4	Advantages and Disadvantages 182
6.3.6.5	Current Challenges and Future Directions 182
6.4	Concluding Remarks 183
	References 183
7	Drug Targeting of the Human Microbiome 191
	Hua Ling, Jee L. Foo, Gourvendu Saxena, Sanjay Swarup,
	and Matthew W. Chang
	Summary 191
7.1	Introduction 191
7.2	The Human Microbiome 192
7.3	Association of the Human Microbiome with Human
	Diseases 194
7.3.1	Nasal – Sinus Diseases 194
7.3.2	Gut Diseases 194
7.3.3	Cardiovascular Diseases 196
7.3.4	Metabolic Disorders 196
7.3.5	Autoimmune Disorders 197
7.3.6	Lung Diseases 197
7.3.7	Skin Diseases 197
7.4	Drug Targeting of the Human Microbiome 198
7.4.1	Prebiotics 198
7.4.2	Probiotics 200
7.4.3	Antimicrobials 201
7.4.3.1	Antibiotics 201
7.4.3.2	Antimicrobial Peptides 202
7.4.4	Signaling Inhibitors 202
7.4.5	Metabolites 203
7.4.5.1	Short-Chain Fatty Acids 203
7.4.5.2	Bile Acids 203
7.4.6	Metabolite Receptors and Enzymes 204
7.4.6.1	Metabolite Receptors 204
7.4.6.2	Metabolic Enzymes 204
7.4.7	Microbiome-Aided Drug Metabolism 205
7.4.7.1	Drug Delivery and Release 205

•	
7.4.7.2	Drug Toxicity 206
7.4.8	Immune Modulators 206
7.4.9	Synthetic Commensal Microbes 207
7.5	Future Perspectives 207
7.6	Concluding Remarks 208
	Acknowledgments 208
	References 209
8	Toward Genome-Scale Models of Signal Transduction
Ū	Networks 215
	Ulrike Münzner, Timo Lubitz, Edda Klipp, and Marcus Krantz
8.1	Introduction 215
8.2	The Potential of Network Reconstruction 219
8.3	Information Transfer Networks 222
8.4	Approaches to Reconstruction of ITNs 225
8.5	The rxncon Approach to ITNWR 230
8.6	Toward Quantitative Analysis and Modeling of Large
0.0	ITNs 234
8.7	Conclusion and Outlook 236
0.7	Acknowledgments 236
	Glossary 237
	References 238
	References 250
9	Systems Biology of Aging 243
	Johannes Borgqvist, Riccardo Dainese, and Marija Cvijovic
	Summary 243
9.1	Introduction 243
9.2	The Biology of Aging 245
9.3	The Mathematics of Aging 249
9.3.1	Databases Devoted to Aging Research 249
9.3.2	Mathematical Modeling in Aging Research 249
9.3.3	Distribution of Damaged Proteins during Cell Division:
	Mathematical Perspective 256
9.3.3.1	Cell Growth 256
9.3.3.2	Cell Death 257
9.3.3.3	Cell Division 257
9.4	Future Challenges 260
	Conflict of Interest 262
	References 262
10	Modeling the Dynamics of the Immune Response 265
10	Elena Abad, Pablo Villoslada, and Jordi García-Ojalvo
10.1	Background 265
10.1	Dynamics of NF-κB Signaling 266
10.2.1	Functional Role and Regulation of NF-kB 266
10.2.1	i unchonal Role and Regulation of INF-KD 200

10.2.2	Dynamics of the NF-κB Response to Cytokine
	Stimulation 267
10.3	JAK/STAT Signaling 273
10.3.1	Functional Roles of the STAT Proteins 273
10.3.2	Regulation of the JAK/STAT Pathway 274
10.3.3	Multiplicity and Cross-talk in JAK/STAT Signaling 275
10.3.4	Early Modeling of STAT Signaling 276
10.3.5	Minimal Models of STAT Activation Dynamics 277
10.3.6	Cross-talk with Other Immune Pathways 279
10.3.7	Population Dynamics of the Immune System 281
10.4	Conclusions 282
	Acknowledgments 283
	References 283
11	Dynamics of Signal Transduction in Single Cells Quantified by
	Microscopy 289
	Min Ma, Nadim Mira, and Serge Pelet
11.1	Introduction 289
11.2	Single-Cell Measurement Techniques 291
11.2.1	Flow Cytometry 291
11.2.2	Mass Cytometry 291
11.2.3	Single-Cell Transcriptomics 292
11.2.4	Single-Cell Mass Spectrometry 292
11.2.5	Live-Cell Imaging 292
11.3	Microscopy 293
11.3.1	Epi-Fluorescence Microscopy 294
11.3.2	Fluorescent Proteins 295
11.3.3	Relocation Sensors 295
11.3.4	Förster Resonance Energy Transfer 298
11.4	Imaging Signal Transduction 300
11.4.1	Quantifying Small Molecules 300
11.4.2	Monitoring Enzymatic Activity 301
11.4.2.1	Endogenous Relocation Sensors 301
11.4.2.2	Passive Relocation Sensors 302
11.4.2.3	Active Relocation Sensors 303
11.4.2.4	FRET Biosensors 304
11.4.3	Probing Protein – Protein Interactions 304
11.4.3.1	FRET in Protein Complexes 304
11.4.3.2	Bimolecular Fluorescence Complementation 305
11.4.3.3	Dimerization-Dependent FP 306
11.4.4	Measuring Protein Synthesis 307
11.4.4.1	mRNA Transcription 307
11.4.4.2	Protein Synthesis 308
11.4.4.3	Expression Dynamics Visualized by Protein
	Relocation 311

11.5	Conclusions 311 References 312
12	Image-Based <i>In silico</i> Models of Organogenesis 319
	Harold F. Gómez, Lada Georgieva, Odysse Michos, and Dagmar Iber Summary 319
12.1	Introduction 319
12.2	Typical Workflow of Image-Based In silico Modeling
	Experiments 320
12.2.1	In silico Models of Organogenesis 322
12.2.2	Imaging as a Source of (Semi-)Quantitative Data 323
12.2.2.1	Imaging a Growing Organ 324
12.2.3	Image Analysis and Quantification 326
12.2.4	Computational Simulations of Models Describing
	Organogenesis 328
12.2.5	Image-Based Parameter Estimation 329
12.2.6	In silico Model Validation and Exchange 329
12.2.6.1	In silico Model Validation 329
12.2.6.2	Model Exchange via the Systems Biology Markup Language
	(SBML) 330
12.3	Application: Image-Based Modeling of Branching
	Morphogenesis 331
12.3.1	Image-Based Model Selection 331
12.4	Future Avenues 334
	References 334
13	Progress toward Quantitative Design Principles of Multicellular
13	Systems 341
	Eduardo P. Olimpio, Diego R. Gomez-Alvarez, and Hyun Youk
	Summary 341
13.1	Toward Quantitative Design Principles of Multicellular
10.1	Systems 341
13.2	Breaking Multicellular Systems into Distinct Functional and Spatial
	Modules May Be Possible 342
13.3	Communication among Cells as a Means of Cell-Cell
	Interaction 346
13.4	Making Sense of the Combinatorial Possibilities Due to Many Ways
	that Cells Can Be Arranged in Space 350
13.5	From Individual Cells to Collective Behaviors of Cell
	Populations 352
13.6	Tuning Multicellular Behaviors 355
13.7	A New Framework for Quantitatively Understanding Multicellular
	Systems 359
	Acknowledgments 361
	References 362

14	Precision Genome Editing for Systems Biology – A Temporal
	Perspective 367
	Franziska Voellmy and Rune Linding
	Summary 367
14.1	Early Techniques in DNA Alterations 367
14.2	Zinc-Finger Nucleases 369
14.3	TALENs 369
14.4	CRISPR-Cas9 370
14.5	Considerations of Gene-Editing Nuclease Technologies 372
14.5.1	Repairing Nuclease-Induced DNA Damage 372
14.5.2	Nuclease Specificity 373
14.6	Applications 376
14.6.1	CRISPR Nuclease Genome-Wide Loss-of-Function Screens
	(CRISPRn) 377
14.6.2	CRISPR Interference: CRISPRi 378
14.6.3	CRISPR Activation: CRISPRa 378
14.6.4	Further Scalable Additions to the CRISPR-Cas Gene Editing Tool
	Arsenal 379
14.6.5	In vivo Applications 379
14.6.5.1	Animal Disease Models 379
14.6.5.2	Gene Therapy 379
14.7	A Focus on the Application of Genome-Engineering Nucleases on
	Chromosomal Rearrangements 380
14.7.1	Introduction to Chromosomal Rearrangements: The First
	Disease-Related Translocation 380
14.7.2	A Global Look at the Mechanisms behind Chromosomal
	Rearrangements 382
14.7.3	Creating Chromosomal Rearrangements Using CRISPR-Cas 383
14.8	Future Perspectives 384
	References 384

Index 393