

CONTENTS

Contributors

xi

Preface

xv

Section I

Biophysical Analysis of Glycans and Glycan Interactions

1. Chemical and Biophysical Approaches for Complete Characterization of Lectin-Carbohydrate Interactions 3

Sabrina Lusvardi, Rodolfo Ghirlando, Jack R. Davison, and Carole A. Bewley

1. Introduction 4
2. Selection and Production of the Lectin 6
3. Selection of the Glycan Target 8
4. Characterization of Lectin-Carbohydrate or Lectin-Glycopeptide Interactions 13
5. Characterization of Lectins Binding to HIV Envelope Glycoprotein gp120 24
6. Characterization of Lectins Binding to and Their Effects on Whole Virions 26
7. Concluding Remarks 31
- References 32

2. Bacterial Surface Glycans: Microarray and QCM Strategies for Glycophenotyping and Exploration of Recognition by Host Receptors 37

Ioanna Kalogralaki, María A. Campanero-Rhodes, Davide Proverbio, Begoña Euba, Junkal Garmendia, Teodor Aastrup, and Dolores Solís

1. Introduction 38
2. Bacteria Culture, Fixation, and Labeling 40
3. Microarray Analysis of Lectin-Bacteria Interactions 43
4. QCM Analysis of Lectin-Bacteria Interactions 54
5. Conclusions 64
- Acknowledgments 68
- References 68

3. Probing Multivalent Protein–Carbohydrate Interactions by Quantum Dot–Förster Resonance Energy Transfer 71

Yuan Guo, W. Bruce Turnbull, and Dejian Zhou

1. Introduction 72
2. Constructing Compact Polyvalent QD–Man Ligands 75
3. Determining Mannose Loading on the QDs and QD–Man Characterization by DLS 84
4. Protein Production and Labeling 86
5. Quantitative FRET Assay, Confirming Mannose–Protein Binding Specificity, Multivalent Interaction, and Investigating Valency on Binding 90
6. Summary 97
- Acknowledgment 97
- References 98

4. Methods for the Detection, Study, and Dynamic Profiling of O–GlcNAc Glycosylation 101

John W. Thompson, Matthew E. Griffin, and Linda C. Hsieh-Wilson

1. Introduction 102
2. Expression and Purification of Y289L GalT 107
3. Labeling of O–GlcNAcylated Proteins With GalNAz 113
4. CuAAC “Click” Reaction With Small-Molecule Alkyne Probes 117
5. CuAAC “Click” Reaction With PEG Alkyne 120
6. Biotin/TAMRA Immunoprecipitation 123
7. O–GlcNAc Site Identification 127
8. Integrated Workflow for Discovery and Biological Assessment of Novel O–GlcNAcylated Proteins 129
9. Conclusions and Future Directions 130
- Acknowledgments 131
- References 131

Section II Glycan Arrays

5. Insights Into Glucan Polysaccharide Recognition Using Glucooligosaccharide Microarrays With Oxime-Linked Neoglycolipid Probes 139

Yan Liu, Angelina S. Palma, Ten Feizi, and Wengang Chai

1. Introduction 140
2. Preparation of Glucooligosaccharide Fractions From Glucan Polysaccharides 142

3. Sequence and Linkage Analysis by Negative-Ion Electrospray Tandem Mass Spectrometry	149
4. Preparation of Oxime-Linked NGLs of Glucooligosaccharides	153
5. Microarray Analyses With NGL-Based Glucooligosaccharide Microarrays	156
6. Discussion and Future Perspectives	161
Acknowledgments	164
References	164

6. Focused Glycomic Profiling With an Integrated Microfluidic Lectin Barcode System

Yuqin Shang and Yong Zeng

1. Introduction	170
2. Methods and Materials	172
3. Results and Discussion	178
4. Conclusion	192
References	193

Section III

Imaging Glycans and Glycan-Active Enzymes

7. Fluorescence-Quenched Substrates for Quantitative Live Cell Imaging of Glucocerebrosidase Activity

Roger A. Ashmus, David L. Shen, and David J. Vocadlo

1. Introduction	200
2. Construction and Evaluation of Probe GBA1-FQ2	202
3. Use of Probe GBA1-FQ2 for Live Cell Imaging	206
4. Summary and Conclusions	213
References	213

8. Activity-Based Probes for Glycosidases: Profiling and Other Applications

Chi-Lin Kuo, Eline van Meel, Kassiani Kytidou, Wouter Willem Kallemijn, Martin Witte, Herman Stephen Overkleeft, Marta Elena Artola, and Johannes Maria Aerts

1. Introduction	218
2. Further Applications of β -Glucosyl Configured Cyclophellitols and the Broadening of the ABP Concept for Other Retaining Glycosidases	223
3. Equipment	225
4. Materials	225

5. Protocol: Fluorescent Detection of MDW933- or MDW941-Labeled GBA on Wet Slab Gels	226
6. In Situ Labeling of GBA for Fluorescence Microscopy	230
7. Conclusion	232
Acknowledgments	232
References	232

9. Covalent Probes for Carbohydrate-Active Enzymes: From Glycosidases to Glycosyltransferases 237

Yong Xu, Najib Uddin, and Gerd K. Wagner	
1. Introduction	238
2. Covalent Probes for Glycosidases and Glycosyltransferases	241
3. Covalent Probes for the Bacterial Glycosyltransferase LgtC	247
4. A Two-Step Protocol for the Fluorescent Labeling of LgtC	252
5. Summary and Conclusion	261
Acknowledgments	262
References	262

10. Revealing the Raft Domain Organization in the Plasma Membrane by Single-Molecule Imaging of Fluorescent Ganglioside Analogs 267

Kenichi G.N. Suzuki, Hiromune Ando, Naoko Komura, Miku Konishi, Akihiro Imamura, Hideharu Ishida, Makoto Kiso, Takahiro K. Fujiwara, and Akihiro Kusumi	
1. Introduction	268
2. Protocols for Incorporating Fluorescent Ganglioside Analogs in the Live-Cell Plasma Membrane	270
3. Single-Molecule Colocalization of Fluorescent Ganglioside Analogs With Raftophilic Receptor CD59 in Various Assembly States	271
4. Protocols for Preparing Cell Specimens for Observing Single-Molecule Colocalization of Fluorescent Ganglioside Analogs With Receptors in the Live-Cell Plasma Membrane	274
5. Protocols for Single-Molecule Colocalization of Fluorescent Ganglioside Analogs With Receptors in the Live-Cell Plasma Membrane	277
6. Conclusions	278
Acknowledgments	279
References	280

11. Intracellular Imaging of Protein-Specific Glycosylation	283
Franziska Doll, Jessica Hassenrück, Valentin Wittmann, and Andreas Zumbusch	
1. Introduction	284
2. Chemical Methods	287
3. Biochemical Methods	293
4. FLIM-FRET Microscopy	304
5. Summary and Conclusion	314
References	315
12. Liposome-Assisted Metabolic Glycan Labeling With Cell and Tissue Selectivity	321
Yifei Du, Ran Xie, Yuting Sun, Xinqi Fan, and Xing Chen	
1. Introduction	322
2. Chemical Synthesis of 9-Azido Sialic Acid and Purification	326
3. Preparation of Ligand-Targeted Liposomes Encapsulating 9AzSia	328
4. LABOR-Enabled Cell-Selective Labeling of Sialoglycans In Vitro	334
5. Targeted Labeling and Imaging of Glycans in the Tumor and Brain	338
6. Proteomic Identification of Sialoglycoproteins in Targeted Tissues by LABOR	343
7. Summary	349
Acknowledgment	349
References	349
13. Imaging Mycobacterial Trehalose Glycolipids	355
Mireille Kamariza, Peyton Shieh, and Carolyn R. Bertozzi	
1. Introduction	355
2. Chemical Synthesis of Trehalose Analogs	358
3. Labeling and Imaging of Mycobacterial Glycolipids	363
4. Conclusion	367
References	367
Author Index	371
Subject Index	393