

Contents

Contributors

ix

Preface

xiii

1. Optical control of protein activity and gene expression by photoactivation of caged cyclofen

1

Fatima Hamouri, Weiting Zhang, Isabelle Aujard, Thomas Le Saux, Bertrand Ducos, Sophie Vríz, Ludovic Jullien, and David Bensimon

- 1. Introduction 2
- 2. The caged-cyclofen-OH/ERT system 5
- 3. Synthesis of caged cyclofen-OH 6
- 4. Protocol for the synthesis of caged cyclofen-OH (cCyc) 7
- 5. Calibration of caged-cyclofen photo-activation 10
- 6. One- and two-photon activation in zebrafish 14
- 7. Transient and permanent gene activation 16
- 8. Conclusions 20
- References 20

2. Reversible optogenetic control of protein function and localization

25

Daniel Z. Wu, Rachel M. Lackner, Chant Aonbangkhen, Michael A. Lampson, and David M. Chenoweth

- 1. Introduction 26
- 2. Equipment and materials 29
- 3. Methods 36
- 4. Analysis 38
- 5. Notes 39
- 6. Summary and conclusions 42
- Acknowledgments 43
- References 43

3. Protocols for the generation of caged guideRNAs for light-triggered RNA-targeting with SNAP-ADARs

47

Alfred Hanswillemenke and Thorsten Stafforst

- 1. Introduction 48
- 2. Materials 50

3. Protocols	53
References	67
4. Combinatorial control of gene function with wavelength-selective caged morpholinos	69
Sankha Pattanayak, Luis Angel Vázquez-Maldonado, Alexander Deiters, and James K. Chen	
1. Introduction	70
2. Design and synthesis of cyclic cMOs	72
3. Microinjection of cMOs into zebrafish embryos	84
4. Photoactivation of cMOs	84
5. Conclusion	86
Acknowledgements	87
References	87
5. Phosphodiester photo-tethers for the (multi-)cyclic conformational caging of oligonucleotides	89
Patricia Müller, Patrick Seyfried, Anton Frühauf, and Alexander Heckel	
1. Introduction	90
2. Photocaging of oligonucleotides	91
3. Installing the photo-tether: Cyclization of oligonucleotides with CuAAC click chemistry	98
4. Protocol	101
5. Melting point studies	107
6. Summary	109
Acknowledgment	109
References	110
6. Chemical modification of proteins with photocleavable groups	113
Karthik Nadendla, Bhagyesh Sarode, and Simon H. Friedman	
1. Introduction	114
2. Methods	115
3. Summary	126
Acknowledgments	127
References	127
7. Intramolecular cross-linking of proteins with azobenzene-based cross-linkers	129
Nobuo Yasuike, Huixin Lu, Peng Xia, and G. Andrew Woolley	
1. Introduction	130

2. Overview of cross-linking strategy	131
3. Cross-linking reaction with IAC and PIP	133
4. Purification	142
5. Identification and characterization	145
6. Conclusions	147
Acknowledgment	148
References	148
8. Ruthenium-cross-linked hydrogels for rapid, visible-light protein release	151
Teresa L. Rapp and Ivan J. Dmochowski	
1. Introduction	151
2. Generating a ruthenium-based photocleavable cross-linker	154
3. Conclusions	163
4. Future directions	164
References	164
9. Chromatically independent, two-color uncaging of glutamate and GABA with one- or two-photon excitation	167
Stefan Passlick and Graham C.R. Ellis-Davies	
1. Introduction	168
2. Methods	176
3. Examples of two-color uncaging	187
4. Summary and conclusion	193
Acknowledgments	194
References	194
10. DIY optogenetics: Building, programming, and using the Light Plate Apparatus	197
Karl P. Gerhardt, Sebastian M. Castillo-Hair, and Jeffrey J. Tabor	
1. Introduction	198
2. Obtaining source files	199
3. Fabrication and assembly	199
4. Initializing a new device	210
5. Using the LPA	218
6. Troubleshooting	225
7. Contribute	225
References	225

11. Pulsatile illumination for photobiology and optogenetics	227
Julia Dietler, Robert Stabel, and Andreas Möglich	
1. Introduction	228
2. Programmable arrays of light-emitting diodes	231
3. Control of bacterial gene expression by varying light intensity and pulse frequency	236
4. Engineering and characterization of photoactivated adenylyl cyclases	240
5. Summary and conclusion	244
Acknowledgments	245
References	245
12. Genetically encoding photoswitchable click amino acids for general optical control of conformation and function of proteins	249
Christian Hoppmann and Lei Wang	
1. Introduction	250
2. Genetic incorporation and application of photoswitchable click amino acids	251
3. Expression and photoregulation of PSCaa-incorporated CaM	255
4. Protocol	257
5. Detecting the photoisomerization of a protein (e.g., CaM)	259
6. Summary	261
Acknowledgment	262
References	262
13. Light-activation of Cre recombinase in zebrafish embryos through genetic code expansion	265
Wes Brown and Alexander Deiters	
1. Introduction	266
2. Expression of photocaged Cre recombinase in zebrafish embryos	268
3. Protocol	270
4. Summary	279
Acknowledgment	279
References	279