

## Contents

Preface XI

List of Contributors XIII

<b>1</b>	<b>The Photoproteins</b>	<b>1</b>
	<i>Osamu Shimomura</i>	
1.1	Discovery of Photoprotein	1
1.2	Various Types of Photoproteins Presently Known	3
1.2.1	Radiolarian (Protozoa) Photoproteins	3
1.2.2	Coelenterate Photoproteins	3
1.2.3	Ctenophore Photoproteins	5
1.2.4	Pholasin ( <i>Pholas</i> Luciferin)	5
1.2.5	<i>Chaetopterus</i> Photoprotein	6
1.2.6	Polynoidin	7
1.2.7	Symplectin	7
1.2.8	<i>Luminodesmus</i> Photoprotein	8
1.2.9	<i>Ophiopsila</i> Photoprotein	8
1.3	Basic Strategy of Extracting and Purifying Photoproteins	9
1.4	The Photoprotein Aequorin	10
1.4.1	Extraction and Purification of Aequorin	10
1.4.1.1	Hydrophobic Interaction Chromatography	11
1.4.2	Properties of Aequorin	11
1.4.2.1	Stability	12
1.4.2.2	Freeze-drying	12
1.4.3	Specificity to Ca <sup>2+</sup>	12
1.4.4	Luminescence of Aequorin by Substances Other Than Divalent Cations	13
1.4.5	Mechanism of Aequorin Luminescence and Regeneration of Aequorin	13
1.4.5.1	Structure of Aequorin	13
1.4.5.2	Luminescence Reaction	14
1.4.5.3	Regeneration	15

*Photoproteins in Bioanalysis*. Edited by Sylvia Daunert and Sapna K. Deo  
Copyright © 2006 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim  
ISBN: 3-527-31016-9

VI | Contents

1.4.6	Inhibitors of Aequorin Luminescence	16
1.4.7	Recombinant Aequorin	16
1.4.8	Semi-synthetic Aequorins	17
1.4.8.1	<i>e</i> -Aequorins	19
	<i>References</i>	20
<b>2</b>	<b>Luminous Marine Organisms</b>	<b>25</b>
	<i>Steven H.D. Haddock</i>	
2.1	Introduction	25
2.1.1	Non-luminous Taxa	28
2.1.2	Luminous Taxa	29
2.2	Taxonomic Distribution of Bioluminescence	29
2.2.1	Bacterial Luminescence	29
2.2.2	Dinoflagellate Luciferin	31
2.2.3	<i>Cypridina (Vargula)</i> Luciferin	32
2.2.4	Coelenterazine	32
2.2.5	Other Luciferins: Known and Unknown	40
2.3	Functions	42
2.3.1	Startle or Distract	42
2.3.2	Burglar Alarm	42
2.3.3	Counterillumination	43
2.3.4	Mating Displays	43
2.3.5	Prey Attraction	43
	<i>References</i>	44
<b>3</b>	<b>Beetle Luciferases:</b>	
	<b>Colorful Lights on Biological Processes and Diseases</b>	<b>49</b>
	<i>Vadim R. Viviani and Yoshihiro Ohmiya</i>	
3.1	Introduction	49
3.2	Beetle Luciferases	50
3.3	Bioanalytical Assays of ATP	51
3.3.1	Biomass Estimation and Microbiological Contamination	53
3.3.2	Cytotoxicity and Cell Viability Tests	54
3.3.3	Enzymatic Assays	54
3.4	Luciferases as Reporter Genes	54
3.4.1	Dual and Multiple Reporter Assays	54
3.5	Biophotonic Imaging in Animals: A Living Light on Diseases	55
3.5.1	Pathogen Infection in Living Models	56
3.5.2	Drug Screening	56
3.5.3	Tumor Proliferation and Regression Studies	57
3.5.4	Gene Delivery and Gene Therapy	57
3.5.5	Luciferase as Biomarkers for Cell Trafficking Studies	57
3.5.6	Immunoassays	57
3.6	Biophotonic Imaging in Plants	58

3.7	Biosensors: Sensing the Environment	58
3.8	Novel Luciferases: Different Colors for Different Occasions	58
	<i>References</i>	60
<b>4</b>	<b>Split Luciferase Systems for Detecting Protein–Protein Interactions in Mammalian Cells Based on Protein Splicing and Protein Complementation</b>	<b>65</b>
	<i>Yoshio Umezawa</i>	
4.1	Introduction	65
4.2	Protein Splicing-based Split Firefly Luciferase System [23]	66
4.2.1	Split Luciferase Works as a Probe for Protein Interaction	68
4.3	Split Renilla Luciferase Complementation System [33]	70
4.3.1	Time Course of the Interaction Between Y941 and SH2n	72
4.3.2	Location of the Interaction Between Y941 and SH2n	72
	<i>References</i>	74
<b>5</b>	<b>Photoproteins in Nucleic Acid Analysis</b>	<b>77</b>
	<i>Theodore K. Christopoulos, Penelope C. Ioannou, and Monique Verhaegen</i>	
5.1	Hybridization Assays	77
5.2	Quantitative Polymerase Chain Reaction	80
5.3	Genotyping of Single-nucleotide Polymorphisms	84
5.4	Conjugation of Aequorin to Oligodeoxynucleotide Probes	87
5.5	Development of New Recombinant Bioluminescent Reporters	89
5.6	Signal Amplification by in Vitro Expression of DNA Reporters Encoding Bioluminescent Proteins	91
5.7	Conclusions	93
	<i>References</i>	93
<b>6</b>	<b>Bioluminescence Resonance Energy Transfer in Bioanalysis</b>	<b>95</b>
	<i>Suresh Shrestha and Sapna K. Deo</i>	
6.1	Introduction	95
6.2	BRET Principle, Efficiency, and Instrumentation	96
6.3	Comparison of BRET and FRET	98
6.4	Examples of BRET Donor–Acceptor Pairs	99
6.5	Applications of BRET in Bioanalysis	102
6.5.1	Homogeneous Assays	102
6.5.2	Protein–Protein Interactions and High-throughput Screening	106
6.6	Conclusions	108
	<i>References</i>	109

<b>7</b>	<b>Photoproteins as <i>in Vivo</i> Indicators of Biological Function</b>	<b>113</b>
	<i>Rajesh Shinde, Hui Zhao, and Christopher H. Contag</i>	
7.1	Overview	113
7.2	Probes Used for <i>in Vivo</i> Bioluminescence Imaging	114
7.3	Probes Used for <i>in Vivo</i> Fluorescence Imaging	116
7.4	Detection Technologies	117
7.5	Current Applications	118
7.5.1	Oncology	118
7.5.2	Infectious Disease	120
7.5.3	Bacterial Infections	121
7.5.4	Viral Infections	123
7.5.5	Viral-mediated Gene Transfer	123
7.5.6	Cell Biology	124
7.5.7	Stem Cell Biology	125
7.6	Protease Sensors	126
7.7	Conclusions	128
	<i>References</i>	128
<b>8</b>	<b>Photoproteins as Reporters in Whole-cell Sensing</b>	<b>131</b>
	<i>Jessika Feliciano, Patrizia Pasini, Sapna K. Deo, and Sylvia Daunert</i>	
8.1	Introduction	131
8.1.1	Biosensors Using Intact Cells	131
8.1.2	Reporter Genes in Genetically Engineered Whole-cell Sensors	132
8.2	The Luciferases	134
8.2.1	Bacterial Luciferases	135
8.2.1.1	<i>luxAB</i> Bioreporters	135
8.2.1.2	<i>luxCDABE</i> Bioreporters	136
8.2.1.3	Naturally Luminescent Bioreporters	136
8.2.2	Eukaryotic Luciferases	136
8.2.2.1	Firefly Luciferase	136
8.2.2.2	Sea Pansy Luciferase	137
8.3	Aequorin	137
8.4	Fluorescent Proteins	138
8.4.1	Green Fluorescent Protein	139
8.4.2	Red Fluorescent Protein	140
8.5	Multiplexing	140
8.6	Applications	141
8.6.1	Stress Factors and Genotoxicants	142
8.6.2	Environmental Pollutants	142
8.6.3	Quorum-sensing Signaling Molecules	144
8.6.4	Antibiotics	145
8.7	Technological Advances	146
	<i>References</i>	148

- 9 Luminescent Proteins in Binding Assays 155**  
*Aldo Roda, Massimo Guardigli, Elisa Michelini, Mara Mirasoli, and Patrizia Pasini*
- 9.1 Introduction 155
  - 9.2 Protein–Protein and Protein–Ligand Interaction Assays 158
    - 9.2.1 FRET and BRET Techniques 158
    - 9.2.2 FRET and BRET Applications 159
    - 9.2.3 Other Detection Principles 163
  - 9.3 Antibody-based Binding Assays 163
    - 9.3.1 Chemical Conjugation 164
    - 9.3.2 Gene Fusion 165
    - 9.3.3 Dual-analyte Assays 166
    - 9.3.4 Expression Immunoassays 166
    - 9.3.5 BRET-based Immunoassays 167
  - 9.4 Biotin–Avidin Binding Assays 168
  - 9.5 Nucleic Acid Hybridization Assays 169
  - 9.6 Other Binding Assays 172
  - 9.7 Concluding Remarks 172
- References 173*
- 10 Luminescent Proteins: Applications in Microfluidics and Miniaturized Analytical Systems 179**  
*Emre Dikici, Laura Rowe, Elizabeth A. Moschou, Anna Rothert, Sapna K. Deo, and Sylvia Daunert*
- 10.1 Miniaturization and Microfluidics 179
  - 10.2 Photoproteins and Applications in Miniaturized Detection Systems 183
    - 10.2.1 Green Fluorescent Protein 184
      - 10.2.1.1 GFP in Miniaturized Microfluidic-based Assays 185
    - 10.2.2 Luciferase 189
      - 10.2.2.1 Luciferase in Miniaturized Microfluidic-based Assays 190
    - 10.2.3 Aequorin 191
      - 10.2.3.1 Aequorin in Miniaturized Microfluidic-based Assays 192
  - 10.3 Future Perspectives 195
- References 196*
- 11 Advances in Instrumentation for Detecting Low-level Bioluminescence and Fluorescence 199**  
*Eric Karplus*
- 11.1 Introduction 199
  - 11.2 Low Light Levels 199
  - 11.3 Methods of Coupling the Signal to the Detector 202
    - 11.3.1 Proximity Focusing 202

x | Contents

11.3.2	Microscope Objectives	203
11.3.3	Macro Lenses	203
11.3.4	Fiber Optics	204
11.4	Evaluating the Performance of an Optical System	205
11.4.1	Numerical Aperture	205
11.4.2	Transmission Efficiency	208
11.4.3	Magnification	209
11.5	Detector Technologies	210
11.6	Selecting the Right Detector	214
11.7	Detector Sensitivity	214
11.8	Detector Noise	216
11.9	Statistics of Photon Counting	220
11.10	Summary	221
	<i>References</i>	223

**12 Photoproteins and Instrumentation:  
Their Availability and Applications in Bioanalysis 225**  
*Leslie Doleman, Stephanie Bachas-Daunert, Logan Davies,  
Sapna K. Deo, and Sylvia Daunert*

Aequorin	225
Obelin	226
Luciferases	227
<i>Aequorea</i> and Anthozoa Fluorescent Proteins	228
Coelenterazines	229
Luminometers	231
Fluorometers	233
Portable Luminometers	234
Disclaimer	234

**Subject Index 235**