

## Contents

<b>1</b>	<b>Summary of Currently Available Mouse Models</b>	<b>1</b>
	<i>Ami Ito, Namiko Ito, Kimie Niimi, Takashi Arai, and Eiki Takahashi</i>	
1.1	Introduction	1
1.2	Origin and History of Laboratory Mice	2
1.3	Laboratory Mouse Strains	3
1.3.1	Wild-Derived Mice	3
1.3.2	Inbred Mice	4
1.3.3	Hybrid Mice	4
1.3.4	Outbred Stocks	8
1.3.5	Closed Colony	8
1.3.6	Congenic Mice	8
1.4	Mutant Mice	9
1.4.1	Spontaneous	9
1.4.2	Transgenesis	9
1.4.3	Targeted Mutagenesis	11
1.4.4	Inducible Mutagenesis	13
1.4.5	<i>Cre-loxP</i> System	13
1.4.6	CRISPR/Cas9 System	15
1.5	Resources of Laboratory Strains	16
1.6	Germ-Free Mice	16
1.7	Gnotobiotic Mice	18
1.8	Specific Pathogen-Free Mice	18
1.9	Immunocompetent and Immunodeficient Mice	18
1.10	Mouse Health Monitoring	19
1.11	Production and Maintenance of Mouse Colony	19
1.11.1	Production Planning	19
1.11.2	Breeding Systems and Mating Schemes	19
1.12	Mating	21
1.13	Gestation Period	21
1.14	Parturition	21
1.15	Parental Behavior and Rearing Pups	21
1.16	Growth of Pups	22
1.17	Reproductive Lifespan	23
1.18	Record Keeping and Colony Organization	23

1.19	Animal Identification	24
1.20	Animal Models in Preclinical Research	24
	References	29
<b>2</b>	<b>General Notes of Chemical Administration to Live Animals</b>	<b>33</b>
	<i>Ami Ito, Namiko Ito, Takashi Arai, Eiki Takahashi, and Kimie Niimi</i>	
2.1	Introduction	33
2.2	Restraint	34
2.2.1	One-Handed Restraint	34
2.2.2	Two-Handed Restraint	34
2.3	Substances	34
2.3.1	Substance Characteristics	34
2.3.2	Vehicle Characteristics	35
2.3.3	Frequency and Volume of Administration	36
2.3.4	Needle Size	37
2.4	Anesthesia	37
2.4.1	Inhaled Agents	38
2.4.2	Injectable Agents	38
2.5	Euthanasia	40
2.6	Administration	41
2.6.1	Enteral Administration	42
2.6.1.1	Oral Administration	42
2.6.1.2	Intragastric Administration	42
2.6.2	Parenteral Administration	42
2.6.2.1	Subcutaneous Administration	44
2.6.2.2	Intraperitoneal Administration	44
2.6.2.3	Intravenous Administration	46
2.6.2.4	Intramuscular Administration	46
2.6.2.5	Intranasal Administration	46
2.6.2.6	Intradermal Administration	46
2.6.2.7	Epicutaneous Administration	46
2.6.2.8	Intratracheal Administration	51
2.6.2.9	Inhalational Administration	51
2.6.2.10	Retro-orbital Administration	52
	References	53
<b>3</b>	<b>Optical-Based Detection in Live Animals</b>	<b>55</b>
	<i>Mikako Ogawa and Hideo Takakura</i>	
3.1	Introduction	55
3.1.1	Basics of Luminescence	55
3.1.2	Appropriate Wavelengths for Live Animal Imaging	56
3.1.3	Advantages and Disadvantages of <i>In Vivo</i> Optical Imaging	58
3.2	Fluorescence Imaging in Live Animals	58
3.2.1	Fluorescent Molecules for Live Animal Imaging	58
3.2.2	How to Detect Fluorescence in Live Animals?	61
3.2.3	Activatable Probes	62
3.2.4	Microscope	68

3.2.5	Application of Fluorescence Imaging to Drug Development	68
3.3	Luminescence Imaging in Live Animals	69
3.3.1	Luminescence Systems for Live Animal Imaging	70
3.3.1.1	Firefly/Beetle Luciferin–Luciferase System	70
3.3.1.2	Coelenterazine-Dependent Luciferase System	76
3.3.1.3	Chemiluminescence System	82
3.3.2	How to Detect Luminescence in Live Animals?	84
3.3.3	Luciferase-Based Bioluminescence Probes for <i>In Vivo</i> Imaging	84
3.4	Summary	87
	References	87
<b>4</b>	<b>Ultrasound Imaging in Live Animals</b>	<b>103</b>
	<i>Francesco Faiva</i>	
4.1	Introduction	103
4.2	High-Frequency Ultrasound Imaging	105
4.3	Ultrasound Contrast Agents	109
4.4	Photoacoustic Imaging	112
4.5	Preclinical Applications	115
4.5.1	Cardiovascular	115
4.5.2	Oncology	120
4.5.3	Developmental Biology	121
	References	123
<b>5</b>	<b>Positron Emission Tomography (PET) Imaging in Live Animals</b>	<b>127</b>
	<i>Xiaowei Ma and Zhen Cheng</i>	
5.1	Introduction	127
5.2	Brief History of PET	128
5.3	Principles of PET	129
5.4	Small-Animal PET Scanners	133
5.5	PET Imaging Tracers	134
5.5.1	Metabolic Probe	134
5.5.2	Specific Receptor Targeting Probe	135
5.5.3	Gene Expression	136
5.5.4	Specific Enzyme Substrate	137
5.5.5	Microenvironment Probe	137
5.5.6	Biological Processes	138
5.5.7	Perfusion Probes	140
5.5.8	Nanoparticles	140
5.6	PET in Animal Imaging	141
5.6.1	PET in Oncology Model	141
5.6.1.1	Cancer Diagnosis	142
5.6.1.2	Personal Treatment Screening	142
5.6.1.3	Therapeutic Effect Monitoring	143
5.6.1.4	Radiotherapy Planning	144
5.6.1.5	Drug Discovery	144
5.6.2	PET in Cardiology Model	145

5.6.3	PET in Neurology Model	146
5.6.4	PET Imaging in Other Disease Models	147
5.7	PET Image Analysis	147
5.8	Outlook for the Future	148
	Reference	149
<b>6</b>	<b>Single-Photon Emission Computed Tomographic Imaging in Live Animals</b>	<b>151</b>
	<i>Yusuke Yagi, Hidekazu Kawashima, Kenji Arimitsu, Koki Hasegawa, and Hiroyuki Kimura</i>	
6.1	Introduction	151
6.2	SPECT Devices Used in Small Animals	152
6.2.1	Innovative Preclinical Full-Body SPECT Imager for Rats and Mice: $\gamma$ -CUBE	155
6.2.2	Innovative Preclinical Full-Body PET Imager for Rats and Mice: $\beta$ -CUBE	156
6.2.3	Innovative Preclinical Full-Body CT Imager for Rats and Mice: X-CUBE	156
6.2.4	Animal Monitoring: Its Importance and Overview of MOLECUBES's Integrated Solution to Advance Physiological Monitoring	157
6.2.5	Selected Applications Acquired on the CUBES	157
6.2.5.1	SPECT Imaging with $\gamma$ -CUBE	158
6.2.5.2	PET Imaging with $\beta$ -CUBE	158
6.2.5.3	CT Imaging with X-CUBE	161
6.3	Characteristics of SPECT Radionuclides and SPECT Imaging Probes	162
6.3.1	Characteristics of SPECT Radionuclides	162
6.3.2	Characteristics of SPECT Imaging Probes	162
6.4	Radiolabeling	163
6.4.1	Characteristic of Radiolabeling	164
6.4.2	Radiolabeling with Technetium-99m	164
6.4.3	Radiolabeling with Iodine-123 and Iodine-131	171
6.4.4	Radioactive Iodine Labeling for Small Molecular Compounds	171
6.4.5	Aromatic Electrophilic Substitution Reaction	171
6.5	<i>In Vivo</i> Imaging of Disease Models	172
6.5.1	Imaging of Central Nervous System Disease	173
6.5.1.1	Alzheimer's Disease	173
6.5.1.2	Parkinson's Disease	174
6.5.1.3	Cerebral Ischemia	176
6.5.2	Imaging of Cardiovascular Disease	177
6.5.2.1	Atherosclerotic Plaque	177
6.5.2.2	Myocardial Ischemia	177
6.5.2.3	Imaging of Cancer	178
6.6	Conclusions	179
	References	180

<b>7</b>	<b>Radiotherapeutic Applications</b>	<b>185</b>
	<i>Koki Hasegawa, Hidekazu Kawashima, Yusuke Yagi, and Hiroyuki Kimura</i>	
7.1	Introduction	185
7.2	Radionuclide Therapy in Tumor-Bearing Mice	186
7.2.1	Radiotherapy with $\beta$ -Emitting Nuclides	186
7.2.2	Radiotherapy Using $\alpha$ -Emitting Nuclides	188
7.3	Radiolabeling Strategy	191
7.3.1	Labeled Target Compounds	191
7.3.2	$^{211}\text{At}$ -Labeled Compounds	192
7.3.3	Chelating Agents for $^{90}\text{Y}$ , $^{177}\text{Lu}$ , $^{225}\text{Ac}$ , $^{213}\text{Bi}$	193
7.3.4	Peptides for Radionuclide Therapy	195
7.3.4.1	Octreotate (TATE) and $[\text{Tyr}^3]$ -Octreotide (TOC)	195
7.3.4.2	NeoBOMB1	196
7.3.4.3	Pentixather	196
7.3.4.4	PSMA-617	196
7.3.4.5	Minigastrin	196
7.3.5	Antibodies for Radionuclide Therapy	197
7.3.5.1	Lintuzumab	197
7.3.5.2	Rituximab	197
7.3.5.3	Trastuzumab	197
7.3.6	Examples of Radiotherapeutic Agents and Target Diseases	197
7.4	Radiotheranostics	200
7.4.1	Radiotheranostics Probe	200
7.4.2	Our Approach to Radiotheranostic Probe Development	202
7.4.3	Expectations and Challenges in Radiotheranostics	202
7.4.4	Boron Neutron Capture Therapy (BNCT)	203
7.4.5	Current Status of BNCT Drugs	204
7.4.5.1	4-Borono-L-Phenylalanine (BPA)	204
7.4.5.2	Sodium Borocaptate (BSH)	204
7.5	Conclusion	205
	References	205
<b>8</b>	<b>Metabolic Glycan Engineering in Live Animals: Using Bio-orthogonal Chemistry to Alter Cell Surface Glycans</b>	<b>209</b>
	<i>Danielle H. Dube and Daniel A. Williams</i>	
8.1	Introduction	209
8.2	Overview of Metabolic Glycan Engineering	210
8.2.1	Origin of Metabolic Glycan Engineering	210
8.2.2	Expansion of the Methodology to Include Unnatural Functional Groups and Bio-orthogonal Elaboration	213
8.3	Bio-orthogonal Chemistries that Alter Cell Surface Glycans	216
8.3.1	Bio-orthogonal Chemistries Amenable to Deployment in Live Animals	216
8.3.2	Bio-orthogonal Chemistries Amenable to Deployment on Cells	221
8.4	Permissive Carbohydrate Biosynthetic Pathways	223

8.4.1	Deployment of Unnatural Monosaccharides in Mammalian Cells	223
8.4.2	Unnatural Sugars that Label Glycans on Bacterial Cells	225
8.5	Cell- and Tissue-Specific Delivery of Unnatural Sugars	226
8.5.1	Harness Inherent Differences in Carbohydrate Biosynthesis	227
8.5.2	Metabolically Label Cells <i>Ex vivo</i> Before Introducing Them <i>In vivo</i>	227
8.5.3	Label Tissues or Organs <i>In vivo</i> Before Analyzing them <i>Ex vivo</i>	229
8.5.4	Employ Tissue-Specific Enzymes to Release Monosaccharide Substrates	229
8.5.5	Deliver Monosaccharide Substrates via Liposomes	231
8.5.6	Use Tissue-Specific Transporters to Induce Monosaccharide Uptake	234
8.6	Applications of Metabolic Glycan Labeling in Mice	234
8.6.1	Imaging Glycans in Mice	234
8.6.2	Covalent Delivery of Therapeutics in Mice	236
8.7	Beyond Mice: Metabolic Glycan Engineering in Diverse Animals	237
8.7.1	Zebra Fish	237
8.7.2	Worms	239
8.7.3	Plants	240
8.8	Conclusions and Future Outlook	240
8.8.1	Metabolic Glycan Engineering Offers a Test Bed for Bio-orthogonal Chemistries	240
8.8.2	New Bio-orthogonal Reactions Could Transform the Field	241
8.8.3	Basic Questions About Glycans Within Living Systems Remain Unanswered	241
	Acknowledgments	241
	References	241
<b>9</b>	<b><i>In Vivo</i> Bioconjugation Using Bio-orthogonal Chemistry</b>	<b>249</b>
	<i>Maksim Royzen, Nathan Yee, and Jose M. Mejia Oneto</i>	
9.1	Introduction	249
9.1.1	IEDDA Chemistry Between <i>trans</i> -Cyclooctene and Tetrazine	249
9.1.2	Synthesis of New Tetrazines and Characterization of Their Reactivity	251
9.1.3	Second Generation of IEDDA Reagents	251
9.1.4	Bond-cleaving Bio-orthogonal “Click-to-Release” Chemistry	251
9.2	<i>In Vivo</i> Applications of IEDDA Chemistry	251
9.2.1	Pretargeting Approach for Cell Imaging	252
9.2.2	Pretargeting Approach for <i>In Vivo</i> Imaging	256
9.2.3	Application of the Pretargeting Strategy for <i>In Vivo</i> Radio Imaging	259
9.2.4	<i>In Vivo</i> Drug Activation Using Bond-cleaving Bio-orthogonal Chemistry	260
9.2.5	Reloadable Materials Allow Local Prodrug Activation	265
9.2.6	Reloadable Materials Allow Local Prodrug Activation Using IEDDA Chemistry	266
9.2.7	Controlled Activation of siRNA Using IEDDA Chemistry	272

- 9.3 Future Outlook 274  
References 277
- 10 In Vivo Targeting of Endogenous Proteins with Reactive Small Molecules 281**  
*Naoya Shindo and Akio Ojida*
- 10.1 Introduction 281
- 10.2 Ligand-Directed Chemical Ligation 282
- 10.2.1 Ligand-Directed Tosyl Chemistry 282
- 10.2.2 Ligand-Directed Acyl Imidazole Chemistry 284
- 10.2.3 Other Chemical Reactions for Endogenous Protein Labeling 287
- 10.3 Labeling Chemistry of Targeted Covalent Inhibitors 287
- 10.3.1 Michael Acceptors 290
- 10.3.2 Haloacetamides 293
- 10.3.3 Activated Esters, Amides, Carbamates, and Ureas 295
- 10.3.4 Sulfur(VI) Fluorides 297
- 10.3.5 Other Warheads and Reactions 300
- 10.4 Conclusion 301  
References 302
- 11 In Vivo Metal Catalysis in Living Biological Systems 309**  
*Kenward Vong and Katsunori Tanaka*
- 11.1 Introduction 309
- 11.2 Metal Complex Catalysts 310
- 11.2.1 Protein Decaging 310
- 11.2.2 Protein Bioconjugation 311
- 11.2.3 Small Molecule – Bond Formation 319
- 11.2.4 Small Molecule – Bond Cleavage 324
- 11.3 Artificial Metalloenzymes 332
- 11.3.1 ArMs Utilizing Naturally Occurring Metals 332
- 11.3.2 ArMs Utilizing Abiotic Transition Metals 335
- 11.4 Concluding Remarks 340  
References 343
- 12 Chemical Catalyst-Mediated Selective Photo-oxygenation of Pathogenic Amyloids 355**  
*Youhei Sohma and Motomu Kanai*
- 12.1 Introduction 355
- 12.2 Catalytic Photo-oxygenation of A $\beta$  Using a Flavin–Peptide Conjugate 357
- 12.3 On–Off Switchable Photo-oxygenation Catalysts that Sense Higher Order Amyloid Structures 358
- 12.4 Near-Infrared Photoactivatable Oxygenation Catalysts: Application to Amyloid Disease Model Mice 363
- 12.5 Closing Remarks 367  
References 368

- 13 Nanomedicine Therapies 373**  
*Patrícia Figueiredo, Flavia Fontana, and Hélder A. Santos*
- 13.1 Introduction 373
- 13.2 Engineering Nanoparticles for Therapeutic Applications 375
- 13.2.1 Physicochemical Properties of NPs 375
- 13.2.2 Surface Functionalization 379
- 13.2.3 Stimuli-Responsive Nanomaterials 381
- 13.2.4 Route of Administration 384
- 13.3 Nanomedicine Platforms 384
- 13.3.1 Lipidic Nanoplatforms 384
- 13.3.2 Polymer-Based Nanoplatforms 389
- 13.3.3 Inorganic Nanoplatforms 391
- 13.3.4 Biomimetic Cell-Derived Nanoplatforms 393
- 13.4 Conclusions 394
- References 395
- 14 Photoactivatable Targeting Methods 401**  
*Xiangzhao Ai, Ming Hu, and Bengang Xing*
- 14.1 Introduction 401
- 14.2 UV Light-Responsive Theranostics 403
- 14.2.1 UV Light-Triggered Photocaged Strategy 403
- 14.2.2 UV Light-Mediated Photoisomerization Strategy 405
- 14.3 Visible Light-Responsive Theranostics 408
- 14.4 Near-Infrared (NIR) Light-Responsive Theranostics 410
- 14.4.1 NIR Light-Mediated Drug Delivery Approach 411
- 14.4.2 NIR Light-Mediated Photodynamic Therapy (PDT) Approach 415
- 14.4.3 NIR Light-Mediated Photothermal Therapy (PTT) Approach 419
- 14.5 Conclusion and Prospects 421
- Acknowledgment 423
- References 423
- 15 Photoactivatable Drug Release Methods from Liposomes 433**  
*Hailey I. Kilian, Dyego Miranda, and Jonathan F. Lovell*
- 15.1 Introduction 433
- 15.1.1 Light-Sensitive Liposomes 434
- 15.2 Mechanisms of Light-Triggered Release from Liposomes 435
- 15.2.1 Light-Induced Oxidation 435
- 15.2.2 Photocrosslinking 436
- 15.2.3 Photoisomerization 438
- 15.2.4 Photocleavage 440
- 15.2.5 Photothermal Release 442
- References 444
- 16 Peptide Targeting Methods 451**  
*Ruei-Min Lu, Chien-Hsun Wu, Ajay V. Patil, and Han-Chung Wu*
- 16.1 Introduction 451
- 16.2 Identification of Targeting Peptides 452



16.2.1	Natural Ligands and Biomimetics	452
16.2.2	Phage Display Peptide Library Screening	454
16.2.3	Synthetic Peptide Library Screening	458
16.3	Therapeutic Applications of Targeting Peptides	460
16.3.1	Therapeutic Peptides	460
16.3.1.1	Naturally Occurring Peptides	464
16.3.1.2	Peptide Conjugates	464
16.3.2	Drug Delivery	465
16.3.2.1	Peptide–Drug Conjugates	465
16.3.2.2	Peptide-Targeted Nanoparticles	467
16.4	Molecular Imaging Mediated by Targeting Peptides	469
16.4.1	Optical Imaging	470
16.4.1.1	Targeting Peptides for Tumor Imaging	471
16.4.1.2	Integrin $\alpha_v\beta_3$ – RGD Tripeptide Targeting Probes:	471
16.4.1.3	Near-Infrared Imaging	472
16.4.2	Positron Emission Tomography	472
16.4.3	Magnetic Resonance Imaging	473
16.5	Summary and Future Perspectives	474
	References	475

**17 Glycan-Mediated Targeting Methods** 489  
*Kenward Vong, Katsunori Tanaka, and Koichi Fukase*

17.1	Introduction	489
17.2	Liver and Liver-Based Disease Targeting	491
17.2.1	Parenchymal Cell Targeting	492
17.2.2	Nonparenchymal Cell Targeting	498
17.3	Immune System Targeting	501
17.3.1	Alveolar Macrophage Targeting	503
17.3.2	Peritoneal Macrophage Targeting	503
17.3.3	Dendritic Cell Targeting	504
17.3.4	Brain Macrophage Targeting	504
17.4	Bacterial Cell Targeting	505
17.5	Cancer Targeting	506
17.5.1	Natural Monosaccharide-Based Methods	506
17.5.2	Synthetic Sugars	508
17.5.3	Complex Glycan Scaffold	511
17.6	Concluding Remarks	514
	References	514

**Index** 531

