

Contents

Preface *xii*

1 Introduction 1

Makoto Oba and Yosuke Demizu

Part I Design 15

2 Classification 17

Makoto Oba

2.1 Introduction 17

2.2 Physicochemical Properties 17

2.3 Chemical Structure 21

2.4 Origin 22

2.5 Cargo 23

3 Arginine-Rich Cell-Penetrating Peptides: Recent Advances of Design and Applications for Intracellular Delivery 29

Ikuhiko Nakase

3.1 Introduction 29

3.2 Arginine-Rich Peptides with Unnatural Amino Acids 30

3.3 β -Hairpin Arginine Peptides for Enhanced Stabilization Under Cytosolic Condition 31

3.4 Cyclic Arginine-Rich Peptides 31

3.5 Cancer-Targeting Cell-Penetrating Peptides 32

3.6 Assembly System Using Arginine-Rich Cell-Penetrating Peptides 33

3.7 Gene Interaction of Arginine-Rich Cell-Penetrating Peptides 34

3.8 Development of Arginine-Rich Cell-Penetrating Peptides for the Delivery of Genes and Oligonucleotides 34

3.9 Molecular Coating with Arginine-Rich Cell-Penetrating Peptides 35

3.10 Enhanced Cytosolic Release using Arginine-Rich Cell-Penetrating Peptides as Additives 36

3.11 Effective Usage of Arginine-Rich Cell-Penetrating Peptides for Extracellular Vesicles, Exosomes 36

3.12	Conclusion	38
	Declaration of Competing Interest	39
	Author Contribution	39
4	Cationic Peptide	45
	<i>Takashi Iwasaki</i>	
4.1	Cationic Peptide	45
4.1.1	Lysine-Rich CPPs	45
4.1.2	Histidine-Rich CPPs	48
5	Amphipathic Peptide	57
	<i>Makoto Oba</i>	
5.1	Introduction	57
5.2	α -Helical Peptides	57
5.3	β -Sheet Peptides	62
5.4	Polyproline Type II Helical Peptides	63
5.5	Others	64
6	Hydrophobic CPPs	69
	<i>Yosuke Demizu</i>	
6.1	MPG	69
6.2	SP	72
6.3	MTS	72
6.4	Hydrophobic MPS	72
6.5	MTS1	72
6.6	TP10	72
6.7	Pep-7	73
6.8	TLM	73
6.9	hCT(9–32)-br	73
6.10	C105Y	73
6.11	M918	74
6.12	PFV	74
6.13	Pept1	74
6.14	hCT(18–32)-br	74
6.15	P4	74
6.16	AA3H	75
6.17	L1-7	75
7	Foldamer	79
	<i>Takuma Kato</i>	
7.1	Introduction	79
7.2	β -Amino Acids	80
7.3	γ -Amino Acids	83
7.4	α,α -Disubstituted Amino Acids	84
7.5	Others	86
7.6	Summary	89

Part II Mechanism 109

- 8 Peptide Structure: Electrophysiological Analysis, Nuclear Magnetic Resonance Analysis, and Molecular Dynamics Simulation of Direct Penetration of Cell-Penetrating Peptides through Bilayer Lipid Membranes 111**
Kayano Izumi, Batsaikhan Mijiddorj, Chihiro Saito, Izuru Kawamura and Ryuji Kawano
- 8.1 Introduction 111
- 8.2 Electrophysiological Analysis of the Direct Penetration of CPPs 113
- 8.2.1 Stable and Reproducible Formation of Planar Bilayer Lipid Membrane using Microfabricated Devices 113
- 8.2.2 Channel Current Signals and Their Classification of Transmembrane Peptides in Planer Bilayer Lipid Membranes 114
- 8.2.3 Channel Current Analysis of the Direct Penetration of CPPs 120
- 8.3 Solid-state NMR Analysis of the Direct Penetration of CPPs 128
- 8.4 Molecular Dynamics Simulation of CPP-Membrane Interactions 129
- 9 Cellular Uptake Mechanisms of Arginine-Rich Cell-Penetrating Peptides 141**
Ikuhiko Nakase
- 9.1 Introduction 141
- 9.2 Endocytosis Mechanisms of Arginine-Rich Cell-Penetrating Peptides 143
- 9.3 Membrane Penetration Mechanisms of Arginine-Rich Peptides 144
- 9.4 Molecular Dynamic Simulation for Understanding Membrane Penetration of Arginine-Rich Cell-Penetrating Peptides 147
- 9.5 Multilamellar Formation of Membranes for Membrane Penetration of Arginine-Rich Peptides 148
- 9.6 Enhanced Membrane Penetration of Arginine-Rich Peptides by Induction of Positive Curvature 148
- 9.7 Internalization Mechanisms into Bacterial Cells 149
- 9.8 Toxicity 149
- 9.9 Importance of Controlled Intracellular Localization of Arginine-Rich Cell-Penetrating Peptides After Uptake for the Delivery of Functional Molecules 150
- 9.10 Conclusion 151
- Declaration of Competing Interest 151
- Author Contribution 152
- 10 Endosomal Escape 159**
Austin R. Prater and Jean-Philippe Pellois
- 10.1 Endosomal Escape 159
- 10.1.1 Introduction 159
- 10.1.2 Endocytosis as a Route of Cell Entry 160
- 10.1.2.1 Types of Endocytosis 160
- 10.1.2.2 The Endocytic Pathway 160
- 10.1.2.3 Endocytosis of CPPs 161

10.1.3	Mechanisms of Endosomal Escape	162
10.1.3.1	Leaky Fusion	162
10.1.3.2	Membrane Destabilization/Permeabilization	165
10.1.3.3	Proton Sponge Effect	166
10.1.4	Is Endosomal Escape Detrimental to Cells?	167
10.1.4.1	Evidence That Endosomal Escape is Toxic	168
10.1.4.2	Evidence That Endosomal Escape is Nontoxic	169
10.1.4.3	Factors That May Dictate Whether Endosomal Escape Is Toxic or Not	170
10.1.5	How to Determine If a CPP Escapes Endosomes?	170
10.1.5.1	Testing for Endosomal Escape by Inhibiting the Endocytic Pathway	170
10.1.5.2	Testing for Endosomal Escape Using Reporters of Membrane Leakage	171
10.1.6	How Can Endosomal Escape Be Improved?	172
10.1.6.1	Direct Modification of CPPs	173
10.1.6.2	Small Molecules	173
10.1.7	Drawbacks of Relying on Endosomal Escape	174
10.1.8	Conclusion	175
11	Pharmacokinetics of Therapeutic and Diagnostic Agents Conjugated with Cell-Penetrating Peptides	183
	<i>Takeshi Fuchigami, Masayuki Munekane and Kazuma Ogawa</i>	
11.1	Introduction	183
11.2	Pharmacokinetics of CPPs	184
11.3	Pharmacokinetics of CPP-Conjugates for CNS Disorders	186
11.4	Pharmacokinetics of CPP-Conjugates for Infectious Diseases	187
11.5	Pharmacokinetics of CPP-Conjugates for Inflammatory Diseases	189
11.6	Pharmacokinetics of CPP-Conjugates for Cancer Treatment	190
11.7	Future Perspective	196
	Part III Delivery Tool	203
12	Drug Delivery	205
	<i>Takashi Misawa</i>	
12.1	Introduction	205
12.2	Delivery by Arg-Rich Peptides	206
12.3	Delivery by Cationic Peptides	209
12.4	Delivery by Amphipathic Peptides	210
12.5	Delivery by Hydrophobic Peptides	211
12.6	Delivery by Foldamers	212
12.6.1	Outlook and Future Direction	212
13	Peptide and Protein Delivery	219
	<i>Hidetomo Yokoo</i>	
13.1	Introduction	219
13.2	The Delivery of Protein by CPPs	224

13.2.1	Covalent Method	224
13.2.1.1	Genetic Method	224
13.2.1.2	Chemical Method	224
13.2.2	Noncovalent Method	228
13.3	The Delivery of Peptides by CPPs	229
13.3.1	Cationic Peptide-Based Method	229
13.3.2	Amphipathic Peptide-Based Method	230
14	Cell-Penetrating Peptides for Nucleic Acid Delivery – An Update	237
	<i>Eric Vivès, Emilie Josse, Karidia Konate, Sébastien Deshayes and Prisca Boisguérin</i>	
14.1	Introduction	237
14.2	Characteristics of CPPs	239
14.3	Mechanism of CPP Internalization	239
14.3.1	Endocytosis-Dependent Mechanism	239
14.3.2	Direct Translocation	241
14.4	CPPs Used as Conjugates for Nucleic Acid Delivery	242
14.4.1	CPP-PNA	242
14.4.2	CPP-PMO	243
14.5	CPPs Used as Nanoparticles for Nucleic Acid Delivery	243
14.5.1	The PepFect Family	245
14.5.2	The WRAP Family	245
14.5.3	The RALA Family	246
14.6	CPP Functionalization for a More Specific Delivery	247
14.6.1	Lipid-Grafting	248
14.6.2	PEGylation	249
14.6.3	Homing Peptide Sequences	250
14.7	Conclusions	250
15	PPMOs: A Case Study for Cell-Penetrating Peptide Application	263
	<i>Scott Bittner, Zoe X. Moulton and Illustrated by Hong M. Moulton</i>	
15.1	Introduction	263
15.2	Peptide Development	267
15.3	Platform Technology	270
15.3.1	Applications of the PPMO Platform: Viruses	270
15.3.2	Applications of the PPMO Platform: Bacteria and Protists	273
15.3.3	Applications of the PPMO Platform: Genetic Disease	275
15.3.3.1	Duchenne Muscular Dystrophy (DMD)	275
15.3.3.2	Spinal Muscular Atrophy (SMA)	276
15.3.3.3	Hutchinson-Gilford Progeria Syndrome (HGPS)	276
15.3.3.4	Cystic Fibrosis	277
15.3.4	Future Applications of the PPMO Platform	277
16	Cell-Penetrating Peptide-Conjugated Polymer Micelle for pDNA/siRNA Delivery	285
	<i>Takanori Kanazawa</i>	
16.1	Introduction	285

16.1.1	Cell-Penetrating Peptide	285
16.1.2	Polymer Micelles	287
16.2	Tat-Conjugated Polymer Micelles	288
16.2.1	Systemic Delivery	288
16.2.1.1	pDNA Delivery	288
16.2.1.2	siRNA Delivery	292
16.2.2	Nose-to-Brain Delivery	294
16.3	Multifunctional Peptide-Conjugated Polymer Micelles	297
16.3.1	Systemic Delivery	297
16.3.1.1	Tumor Tissue	297
16.3.1.2	Inflamed Tissue	301
16.3.2	Oral Delivery	304
16.4	Conclusions and Future Perspective	307
17	Lipid-Based Nanoparticles Using Cell-Penetrating Peptides	315
	<i>Ikramy A. Khalil and Hideyoshi Harashima</i>	
17.1	Introduction	315
17.2	Cell-Penetrating Peptides	316
17.2.1	Overview	316
17.2.2	Types	317
17.2.3	Mechanism of Cellular Internalization	317
17.3	Lipid-based Nanoparticles	320
17.3.1	Overview	320
17.3.2	Types	320
17.4	CPP-Modified Lipid Nanoparticles	321
17.4.1	CPP-LNPs for Cellular Internalization	321
17.4.2	CPP-LNPs for Tumor Targeting	322
17.4.3	CPP-LNPs for Liver Targeting	323
17.4.4	Other Applications of CPP-LNPs	323
17.5	Improving CPP-LNPs	324
17.6	Conclusion	324
	Part IV Application	329
18	Cell-Penetrating Peptides as Tools to Facilitate Oral Delivery of Biopharmaceuticals	331
	<i>Camilla Jantzen Asgreen, Freja Fredholt, Hanne Mørck Nielsen and Mie Kristensen</i>	
18.1	Introduction	331
18.2	Challenges for Oral Delivery of Biopharmaceuticals	331
18.3	Cell-Penetrating Peptides as Carriers for Oral Delivery of Biopharmaceuticals	333
18.3.1	Parameters Affecting the Potency of Cell-Penetrating Peptides as Carriers for Transepithelial Cargo Absorption	333
18.3.2	Formulation of Cell-Penetrating Peptides	342
18.3.3	Region-Dependent CPP-Mediated Intestinal Absorption of Biopharmaceuticals	342

18.4	In vivo Methods and Data Reporting when Studying Intestinal Absorption of Biopharmaceuticals	343
18.4.1	Oral Gavage	343
18.4.2	In situ Administration	344
18.4.3	Intestinal Injection	344
18.4.4	Experimental Readout	344
18.4.5	Safety of Oral Administration of Cell-Penetrating Peptides	345
18.4.6	Reporting and Statistical Analysis of Data from in vivo Studies	346
18.5	Mechanism of Cell-Penetrating Peptide-Mediated Cargo Delivery Across the Intestinal Barrier	347
18.5.1	Mucus as Barrier to Cell-Penetrating Peptide-Mediated Cargo Delivery Across the Intestine	347
18.5.2	Mechanism of Transepithelial Transport of Cell-Penetrating Peptides and Their Cargo	347
18.6	Perspectives	349
19	"Intranasal Delivery"	355
	<i>Noriyasu Kamei and Mariko Takeda-Morishita</i>	
19.1	Enhanced Systemic Absorption of Biologics by Coadministration with CPPs	355
19.2	Enhanced Nose-to-Brain Delivery of Biologics by Coadministration with CPPs	357
19.3	Mechanistic Insight for Enhanced Nose-to-Brain Drug Delivery with CPPs	358
19.4	Therapeutic Effects After Enhanced Nose-to-Brain Delivery of Biologics	360
19.5	Perspectives	362
20	Clinical Trials	367
	<i>Makoto Oba</i>	
20.1	Introduction	367
20.2	Peptides	367
20.3	Proteins	373
20.4	Low-Molecular-Weight (LMW) Drugs	374
20.5	Nucleic Acids	375
20.6	Fluorescent Dye	376
21	Plant	381
	<i>Kousuke Tsuchiya and Keiji Numata</i>	
21.1	Introduction	381
21.2	CPP Design and Availability for Plant Cells	382
21.3	Artificial CPP for Plant Cells	383
21.4	Internalization Mechanism	386
21.5	Polypeptide-Based Carriers for Material Delivery	389
21.6	Perspective	393
	Index	397