# 1

# Introduction

*Makoto Oba[1] and Yosuke Demizu[2]*

[1] *Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kyoto, Japan*
[2] *National Institute of Health Sciences, Division of Organic Chemistry, Kanagawa, Japan*

Cell-penetrating peptides (CPPs) are short peptides (in general composed of 5–30 amino acids) that can be efficiently internalized into cells and have great potential for the delivery of membrane-impermeable bioactive molecules into cells. The CPP is sometimes called a protein transduction domain (PTD) or Trojan peptide. In 1988, such a peptide was first discovered in human immunodeficiency virus type 1 (HIV-1) protein transactivator of transcription (Tat). Since then, numerous sequences of CPPs have been discovered in natural peptides/proteins such as Tat peptide and CPPs have also been artificially designed. Furthermore, the cell-penetrating mechanisms of CPPs in vitro and in vivo have been investigated, and CPPs have been used as delivery tools for drugs, peptides, proteins, nucleic acids, and nanoparticles. Several CPPs are currently under investigation in clinical trials. More than 500 research papers per year have been published since 2012 with a keyword of "CPP" or "PTD," and numbers have reached slightly less than 1000 for the last few years. From these numbers, we can understand the usefulness and significance of CPPs.

This book describes the design, mechanism, delivery tools, and applications of CPPs. There are several books and journals' special issues on CPPs. However, none has previously categorized such topics. On the topic of design, the classification of CPPs is first described based on their characteristics, and then, typical types of CPPs (cationic, amphipathic, and hydrophobic peptides) and Arg-rich peptides and foldamers are introduced. The topic of mechanism deals with important factors of CPPs, such as peptide secondary structure, cellular uptake, endosomal escape, and pharmacokinetics in vivo. The topic of delivery tools is categorized based on cargos, drugs, peptides and proteins, nucleic acids, and morpholino oligomers. Furthermore, CPPs assisting in the efficient delivery of nano-sized drug delivery systems, polymeric micelles, and lipid-based nanoparticles are introduced as delivery tools. The final topic of applications covers oral delivery and intranasal delivery using CPPs, CPPs in clinical trials, and applications in plants. Table 1.1 lists the representative

*Cell-Penetrating Peptides: Design, Development and Applications*, First Edition. Makoto Oba and Yosuke Demizu.  Published 2023 by WILEY-VCH GmbH.

CPPs introduced in this book. We hope that this book will be useful for readers studying and treating CPPs now and in the future.

**Table 1.1** Lists of CPPs introduced in this book.

| CPP | Sequence | Ref. |
|---|---|---|
| [C12-R4] | Cyclic(CXRRRR)<br>X: (*R*,*S*)-2-amino tetradecanoic acid | [1] |
| [R6W3] | Cyclic(CRRWWRRWRR) | [1] |
| A2-17 | LRKLRKRLLRLWKLRKR | [2] |
| A2-17KR | LRRLRRRLLRLWRLRRR | [2] |
| AA3H | MASIWVGHRG | [3] |
| $Ac_5c^{NH2}$ peptide | $(RRX)_3$<br>X: $Ac_5c^{NH2}$, 1,3-diaminocyclopentanecarboxylic acid | [4] |
| $Ac_5c^{Gu}$ peptide | $(RRX)_3$<br>X: $Ac_5c^{Gu}$, 1-amino-3-guanidinocyclopentanecarboxylic acid | [4] |
| $Ac_6c^{NH2}$ peptide | $(RRX)_3$<br>X: $Ac_6c^{NH2}$, 1,4-diaminocyclohexanecarboxylic acid | [5] |
| Amphipathic β-peptide | $[(S,S)$-ACHC-$\beta^3$hArg-$\beta^3$hArg$]_3$<br>ACHC: *trans*-2-aminocyclohexanecarboxylic acid<br>$\beta^3$hArg: $\beta^3$-homoarginine | [6] |
| $Api^{C2Gu}$ peptide | $(RRX)_3$<br>X: $Api^{C2Gu}$, 4-aminopiperidine-4-carboxylic acid derivative | [7] |
| αR7W2 | RRRWRRWRR | [8] |
| ARF (1-22) | MVRRFLVTLRIRRACGPPRVRV | [9] |
| ARF (19-31) | RVRVFVVHIPRLT | [9] |
| β-Heptaarginine | $(\beta^3$hArg$)_7$<br>$\beta^3$hArg: $\beta^3$-homoarginine | [10] |
| β-Heptalysine | $(\beta^3$hLys$)_7$<br>$\beta^3$hLys: $\beta^3$-homolysine | [10] |
| β-Tat | $\beta^3$hArg-$(\beta^3$hLys$)_2$-$(\beta^3$hArg$)_2$-$\beta^3$hGln-$(\beta^3$hArg$)_3$ | [11] |
| $Bac_{15\text{-}24}$ | RRIRPRPPRLPRPRPRPLPFPRPG | [12] |
| Bip2 | VPTLK | [13] |
| Block3 | LLULLULLUGGGRRRRRRRRR<br>U: Aib, 2-aminoisobutyric acid | [14] |
| BP100 | KKLFKKKKILKYL | [15] |
| bPrPp (1-30) | MVKSKIGSWILVLFVAMWSDVGLCKKRPKP | [16] |
| Butyl-TH | AGYLLGH$_B$INLH$_B$H$_B$LAH$_B$LUH$_B$H$_B$IL<br>U: Aib, 2-aminoisobutyric acid; H$_B$: 3-butylhistidine | [17] |
| C105Y | CSIPPEVKFNKPFVYLI | [18] |
| CADY | GLWRALWRLLRSLWRLLWRA-cysteamide | [19] |
| CH2R4H2C | CHHRRRRHHC | [20] |

*(Continued)*

**Table 1.1** (Continued)

| CPP | Sequence | Ref. |
|---|---|---|
| cLK | Cyclic Ac-CKKLLKLLKKLLKLGGLKKLLKLLKKLLKLLK<br>Crosslink between the side chains of C and K | [21] |
| CPP12 | Cyclic($FXR_4$)<br>X: L-2-naphthylalanine | [22] |
| CPP2 | DSLKSYWYLQKFSWR | [23] |
| CPP44 | KRPTMRFRYTWNPMK | [23] |
| CPP9 | Cyclic(fXRrRrQ)<br>X: L-2-naphthylalanine | [24] |
| Cyclic [$W(RW)_4$] | Cyclic[$W(RW)_4$] | [25] |
| Cyclic $R_9$ | Cyclic(CRRRRRRRRR) | [26] |
| Cyt $c^{77\text{-}101}$ | GTKMIFVGIKKKEERADLIAYLKKA | [27] |
| D-Oligoarginine | $r_n$ | [28] |
| D-$Tat^{49\text{-}57}$ | Rkkrrqrrr | [28] |
| DPV1047 | VKRGLKLRHVRPRVTRMDV | [29] |
| DPV3 | RKKRRRESRKKRRRES | [29] |
| dTat-Sar-EED4 | rrrqrrkkrXXXXXXGWWG<br>X: Sar, sarcosine | [30] |
| FHV $coat^{35\text{-}49}$ | RRRRNRTRRNRRRVR | [31] |
| GALA | WEAALAEALAEALAEHLAEALAEALEALAA | [32] |
| H5WYG | GLFHAIAHFIHGGWHGLIHGWYG | [33] |
| HA2 | GDIMGEWGNEIFGAIAGFLG | [34] |
| HAad | IWLTALKFLGKAAAKAXAKQXLSKL<br>X: L-2-aminoadipic acid | [35] |
| $hCT^{9\text{-}32}$-br | LGTYTQDFNK(X)FHTFPQTAIGVGAP<br>X: PKKKRKVEDPGVGFA | [36] |
| HIV-1 $Rev^{34\text{-}50}$ | TRQARRNRRRRWRERQR | [31] |
| HL | CHHHHHRRWQWRHHHHHC | [37] |
| HR9 | CHHHHHRRRRRRRRRHHHHHC | [38] |
| HTLV-II $Rex^{4\text{-}16}$ | TRRQRTRRARRNR | [31] |
| Hydrophobic MPS | VTVLAGALAGVGVG | [39] |
| K10H16 | KKKKKKKKGHHHHHHHHHHHHHHHH | [40] |
| KAibA | poly(KUA)<br>U: Aib, 2-aminoisobutyric acid | [41] |
| KALA | WEAKLAKALAKALAKHLAKALAKALKACEA | [42] |
| KLA | $(KLAKLAK)_2$ | [43] |
| KLA10 | KALKKLLAKWLAAAKALL | [44] |

*(Continued)*

**Table 1.1** (Continued)

| CPP | Sequence | Ref. |
|---|---|---|
| L-Pro$^{Gu}$ peptide | $(RRX)_3$<br>X: L-Pro$^{Gu}$; 4-guanidinoproline | [45] |
| L1-7 | Cyclic FIDIIIKILLI<br>Crosslink between the side chains of D and K | [46] |
| L17E | IWLTALKFLGKHAAKHEAKQQLSKL | [47] |
| L6 | RRWQWR | [48] |
| LAH4 | KKALLALALHHLAHLALHLALALKKA | [49] |
| LAH4-L1 | KKALLAHALHLLALLALHLAHALKKA | [50] |
| LH | LHHLLHHLHHLLHH | [51] |
| LK | LKKLLKLLKKLLKL | [52] |
| LTP | RRKRRKKRRKRRKKKAC | [53] |
| M1 | TFYGGRPKRNNFLRGIR | [54] |
| M918 | MVTVLFRRLRIRRACGPPRVRV | [55] |
| MAP | KLALKLALKALKAALKLA | [56] |
| MAP(Aib) | KLULKLULKULKAULKLU<br>U: Aib, 2-aminoisobutyric acid | [57] |
| Melittin | GIGAVLKVLTTGLPALISWIKRKRQQ | [58] |
| Mitoparan | INLKKLAKLUKKIL<br>U: Aib, 2-aminoisobutyric acid | [59] |
| MPG | GALFLGFLGAAGSTMGAWSQPKKKRKV | [60] |
| MTS | AAVALLPAVLLALLAP | [61] |
| MTS1 | AAVLLPVLLAAP | [62] |
| N-hxg9 | $(N\text{-hxg})_9$<br>*N*-hxg: *N*-guanidinohexylglycine | [28] |
| NF51 | δ-(Stearyl-AGYLLG)OINLKALAALAKKIL<br>O: Orn, L-ornithine | [63] |
| NF55 | δ-(Stearyl-AGYLLG)OINLKALAALAKAIL<br>O: Orn, L-ornithine | [64] |
| NF70 | δ-(Arachidyl-HHHHYHHG)OILLKALKALAKAIL<br>O: Orn, L-ornithine | [65] |
| Oligoarginine | $R_n$ | [28, 31] |
| Oligohistidine | $H_n$ | [66] |
| Oligolysine | $K_n$ | [67] |
| Oligourea3 | iPrNHCO-VUHUWUVUHUWURUγV<br>γV: γ-valine | [68] |
| p18 | LSTAADMQGVVTDGMASG | [69] |

*(Continued)*

**Table 1.1** (Continued)

| CPP | Sequence | Ref. |
|---|---|---|
| P4 | LGAQSNF | [70] |
| PenetraMax | KWFKIQMQIRRWKNKR | [71] |
| Penetratin | RQIKIWFQNRRMKWKK | [72] |
| Pep-1 | KETWWETWWTEWSQPKKKRKV | [73] |
| Pep-7 | SDLWEMMMVSLACQY | [74] |
| Pept1 | PLILLRLLRGQF | [75] |
| PF14 | Stearyl-AGYLLGKLLOOLAAAALOOLL<br>O: Orn, L-ornithine | [76] |
| PF3 | Stearyl-AGYLLGKINLKALAALAKKIL | [77] |
| PF6 | Stearyl-AGYLLGK(X)INLKALAALAKKIL<br>X: 4-trifluoromethylquinoline-based derivative | [78] |
| PFV | PFVYLI | [79] |
| PG-1 | RGGRLCYCRRRFCVCVGR | [80] |
| pHLIC | Cyclic(EEEEWWWWWC) | [81] |
| pHLIP | AAEQNPIYWWARYADWLFTTPLLLLDLALLVDADEGTCG | [82] |
| Pip6a | RXRRBRRXRYQFLIRXRBRXRB<br>X: aminohexanoyl; B: β-alanine | [83] |
| ppTG1 | GLFKALLKLLKSLWKLLLKA | [84] |
| ppTG20 | GLFRALLRLLRSLWRLLLRA | [84] |
| PR20 | PRPRPRPRPRPRPRPRPRPRPRPRPRPRPRPRPRPR | [85] |
| PreS2-TLM | PLSSIFSRIGDP | [86] |
| PTD4 | YARAAARQARA | [87] |
| pVEC | LLIILRRRIRKQAHAHSK | [88] |
| $R_6/W_3$ | RRWWRRWRR | [89] |
| $R_9F_2C$ | RRRRRRRRRFFC | [90] |
| RGE | RGERPPR | [91] |
| RICK | Kwllrwlsrllrwlarwlg | [92] |
| RLA | Rlarlarrlarlar | [93] |
| RRU peptide | $(RRU)_n$ (n = 1–6)<br>U: Aib, 2-aminoisobutyric acid | [94] |
| RRX peptide | $(RRX)_3$<br>X: (*S*)-α-methylleucine, 1-aminocyclopentanecarboxylic acid, or (3*S*,4*S*)-1-amino-3,4-dimethoxycyclopentanecarboxylic acid | [95] |
| RW16 | RRWRRWWRRWWRRWRR | [96] |
| RWRWR | RWVRVpGOWIRQ<br>O: Orn, L-ornithine | [97] |

*(Continued)*

**Table 1.1** (Continued)

| CPP | Sequence | Ref. |
|---|---|---|
| RXR | (R-Ahx-R)$_4$-Ahx-(β-Ala)<br>Ahx: 6-aminohexanoic acid; β-Ala: β-alanine | [98] |
| SAP | VRLPPPVRLPPPVRLPPP | [99] |
| SAP(E) | VELPPPVELPPPVELPPP | [100] |
| sC18 | GLRKRLRKFRNKIKEK | [101] |
| SLP $F_4R_4$ | FFFFRRRR | [102] |
| SLP $R_2F_4R_2$ | RRFFFFRR | [102] |
| SP | RPKPQQFGLM | [103] |
| Stearyl-oligoarginine | Stearyl-$R_n$ | [104, 105] |
| Stitched peptide | $\underline{X_1}$RRR$\underline{X_2}$RRRRRR$\underline{X_3}$<br>$X_1$: (*S*)-2-(4-pentenyl)alanine; $X_2$: 2,2-bis-(4-pentenyl)glycine; $X_3$: (*S*)-2-(7-octenyl)alanine<br>Crosslinks between the side chains of $\underline{X_1}$ and $\underline{X_2}$ and $\underline{X_2}$ and $\underline{X_3}$ | [106] |
| SVS-1 | KVKVKVKVpPTKVKVKVK | [107] |
| SynB1 | RGGRLSYSRRRFSTSTGR | [108] |
| Tachyplesin | KWCFRVCYRGICYRRCR | [109] |
| $Tat^{48-60}$ | GRKKRRQRRRPPQ | [110] |
| $Tat^{49-57}$ | RKKRRQRRR | [110] |
| TH | AGYLLGHINLHHLAHLUHHIL<br>U: Aib, 2-aminoisobutyric acid | [111] |
| TK | AGYLLGKINLKKLAKLUKKIL<br>U: Aib, 2-aminoisobutyric acid | [112] |
| TLM | PLSSIFSRIGDP | [113] |
| TP10 | AGYLLGKINLKALAALAKKIL | [114] |
| Transportan | GWTLNSAGYLLGKINLKALAALAKKIL | [115] |
| VP22 | DAATATRGRSAASRPTQRPRAPARSASRPRRPVE | [116] |
| $Vpr^{55-82}$ | TGVEALIRILQQLLFIHFRIGCRHSRIG | [117] |
| VT5 | DPKGDPKGVTVTVTVTVTGKGDPKPD | [118] |
| WRAP1 | LLWRLWRLLWRLWRLL | [119] |
| WRAP5 | LLRLLRWWWRLLRLL | [119] |

## References


1 Amoura, M., Illien, F., Joliot, A. et al. (2019). Head to tail cyclisation of cell-penetrating peptides: impact on GAG-dependent internalization and direct translocation. *Chemical Communications* 55: 4566–4569.

2 Ohgita, T., Takeuchi-Haraya, Y., and Nadai, R. (2019). A novel amphipathic cell-penetrating peptide based on the N-terminal glycosaminoglycan binding region of human apolipoprotein E. *BBA – Biomebnranes* 1861: 541–549.

3 Kim, H.Y., Yum, S.Y., Jang, G. et al. (2015). Discovery of a non-cationic cell penetrating peptide derived from membrane-interacting human proteins and its potential as a protein delivery carrier. *Scientific Reports* 5: 11719.

4 Kato, T., Oba, M., Nishida, K. et al. (2018). Cell-penetrating peptides using cyclic α,α-disubstituted α-amino acids with basic functional groups. *ACS Biomaterials Science & Engineering* 4: 1368–1376.

5 Kato, T., Kita, Y., Iwanari, K. et al. (2021). Synthesis of six-membered carbocyclic ring α,α-disubstituted amino acids and arginine-rich peptides to investigate the effect of ring size on the properties of the peptide. *Bioorganic & Medicinal Chemistry* 38: 116111.

6 Potocky, T.B., Menon, A., and Gellman, S.H. (2005). Effects of conformational stability and geometry of guanidinium display on cell entry by β-peptides. *Journal of the American Chemical Society* 127: 3686–3687.

7 Yamashita, H., Oba, M., Misawa, T. et al. (2016). A helix-stabilized cell-penetrating peptide as an intracellular delivery tool. *ChemBioChem* 17: 137–140.

8 Walrant, A., Bauza, A., Girardet, C. et al. (1862). Ionpair-π interactions favor cell penetration of arginine/tryptophan-rich cell-penetrating peptides. *BBA – Biomembranes* 2020: 183098.

9 Johansson, H., El-Andaloussi, S., Holm, T. et al. (2008). Characterization of a novel cytotoxic cell-penetrating peptide derived from p14ARF protein. *Molecular Therapy* 16 (1): 115–123.

10 Rueping, M., Mahajan, Y., Sauer, M. et al. (2002). Cellular uptake studies with β-peptides. *ChemBioChem* 3: 257–259.

11 Umezawa, N., Gelman, M.A., Haigis, M.C. et al. (2002). Translocation of a β-peptide across cell membranes. *Journal of the American Chemical Society* 124 (3): 368–369.

12 Sadler, K., Eom, K.D., Yang, J.-L. et al. (2002). Translocating proline-rich peptides from the antimicrobial peptide bactenecin 7. *Biochemistry* 41: 14150–14157.

13 Gomez, J.A., Gama, V., Yoshida, T. et al. (2007). Bax-inhibiting peptides derived from Ku70 and cell-penetrating pentapeptides. *Biochemical Society Transactions* 35 (4): 797–801.

14 Misawa, T., Ohoka, N., Oba, M. et al. (2019). Development of 2-aminoisobutyric acid (Aib)-rich cell-penetrating foldamers for efficient siRNA delivery. *Chemical Communications* 55: 7792–7795.

15 Badosa, E., Ferre, R., Planas, M. et al. (2007). A library of linear undecapeptides with bactericidal activity against phytopathogenic bacteria. *Peptides* 28: 2276–2285.

16 Biverstahl, H., Andersson, A., Graslund, A. et al. (2004). NMR solution structure and membrane interaction of the N-terminal sequence (1-30) of the bovine prion protein. *Biochemistry* 43: 14940–14947.

17 Yao, J., Ma, Y., Zhang, W. et al. (2007). Design of new acid-activated cell-penetrating peptides for tumor drug delivery. *Peer Journal* 5: e3429.

18 Rhee, M. and Davis, P. (2006). Mechanism of uptake of C105Y, a novel cell-penetrating peptide. *The Journal of Biological Chemistry* 281 (2): 1233–1240.

19 Crombez, L., Aldrian-Herrada, G., Konate, K. et al. (2009). A new potent secondary amphipathic cell-penetrating peptide for siRNA delivery into mammalian cells. *Molecular Therapy* 17 (1): 95–103.

**20** Tanaka, K., Kanazawa, T., Ogawa, T. et al. (2010). Disulfide crosslinked stearoyl carrier peptides containing histidine enhance siRNA uptake and gene silencing. *International Journal of Pharmaceutics* 398: 219.

**21** Nam, S.H., Lee, Y., Ahn, J.H. et al. (2020). Augmented osteogenesis of mesenchymal stem cells using a fragmented Runx2 mixed with cell-penetrating, dimeric a-helical peptide. *European Journal of Pharmaceutical Sciences* 144: 105210.

**22** Cerulli, R.A., Shehaj, L., Tosic, I. et al. (2020). Cytosolic delivery of peptidic STAT3 SH2 domain inhibitors. *Bioorganic & Medicinal Chemistry* 28: 115542.

**23** Kondo, E., Saito, K., Tashiro, Y. et al. (2012). Tumour lineage-homing cell-penetrating peptides as anticancer molecular delivery systems. *Nature Communications* 3: 951.

**24** Salim, H., Song, J., Sahni, A. et al. (2020). Development of a cell-permeable cyclic peptidyl inhibitor against the Krap1-Nrf2 interaction. *The Journal of Organic Chemistry* 85: 1416–1424.

**25** Shirazi, N.A., Tiwari, R., Chhikara, B.S. et al. (2013). Design and biological evaluation of cell-penetrating peptide-doxorubicin conjugates as prodrugs. *Molecular Pharmaceutics* 10: 488–499.

**26** Uhl, P., Grundmann, C., Sauter, M. et al. (2020). Coating of PLA-nanoparticles with cyclic, arginine-rich cell penetrating peptides enables oral delivery of liraglutide. *Nanomedicine: Nanotechnology, Biology, and Medicine* 24: 102132.

**27** Howl, J. and Jones, S. (2008). Proteomimetic cell penetrating peptides. *International Journal of Peptide Research and Therapeutics* 14: 359–366.

**28** Wender, P.A., Mitchell, D.J., Pattabiraman, K. et al. (2000). The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: peptoid molecular transporters. *Proceedings of the National Academy of Sciences of the United States of America* 97 (24): 13003–13008.

**29** De Coupade, C., Fittipald, A., Chagnas, V. et al. (2005). Novel human-derived cell-penetrating peptides for specific subcellular delivery of therapeutic biomolecules. *Biochemical Journal* 390: 407–418.

**30** Miyamoto, T., Tsuchiya, K., and Numata, K. (2020). Dual peptide-based gene delivery system for the efficient transfection of plant callus cells. *Biomacromolecules* 21: 2735–2744.

**31** Futaki, S., Suzuki, T., Ohashi, W. et al. (2001). Arginine-rich peptides. *The Journal of Biological Chemistry* 276 (8): 5836–5840.

**32** Subbarao, N.K., Parente, R.A., Szoka, F.C. et al. (1987). pH-Dependent bilayer destabilization by an amphipathic peptide. *Biochemistry* 26: 2964–2972.

**33** Midoux, P., Kichler, A., Boutin, V. et al. (1998). Membrane permeabilization and efficient gene transfer by a peptide containing several histidines. *Bioconjugate Chemistry* 9: 260–267.

**34** Wadia, J.S., Stan, R.V., and Dowdy, S.F. (2004). Transducible TAT-HA fusogenic peptide enhances escape of TAT-fusion proteins after lipid raft micropinocytosis. *Nature Medicine* 10 (3): 310–315.

**35** Sakamoto, K., Michibata, J., Hirai, Y. et al. (2021). Potentiating the membrane interaction of an attenuated cationic amphipathic lytic peptide for intracellular protein delivery by anchoring with pyrene moiety. *Bioconjugate Chemistry* 32: 950–957.

**36** Rennert, R., Wespe, C., Beck-Sickinger, A.G. et al. (2006). Developing novel hCT derived cell-penetrating peptides with improved metabolic stability. *Biochimica et Biophysica Acta* 1758: 347–354.

**37** Lee, H.-J., Huang, Y.-W., Chiou, S.-H. et al. (2019). Polyhistidine facilitates direct membrane translocation of cell-penetrating peptides into cells. *Scientific Reports* 9: 9398.

**38** Liu, M.-J., Chou, J.-C., Lee, H.-J. et al. (2013). A gene delivery method mediated by three arginine-rich cell-penetrating peptides in plant cells. *Advanced Studies in Biology* 5 (2): 71–88.

**39** Zhang, L., Torgerson, T.R., Liu, X.-Y. et al. (1998). Preparation of functionally active cell-permeable peptides by single-step ligation of two peptide modules. *Proceedings of the National Academy of Sciences of the United States of America* 95: 9184–9189.

**40** Iwasaki, T., Murakami, N., and Kawano, T. (2020). A polylysine-polyhistidine fusion peptide for lysosome-targeted protein delivery. *Biochemical and Biophysical Research Communications* 533: 905–912.

**41** Terada, K., Gimenez-Dejoz, J., Miyagi, Y. et al. (2020). Artificial cell-penetrating peptide containing periodic α-aminoisobutyric acid with long-term internalization efficiency in human and plant cells. *ACS Biomaterials Science & Engineering* 6: 3287–3298.

**42** Wyman, T.B., Nicol, F., Zelphati, O. et al. (1997). Design, synthesis, and characterization of a cationic peptide that binds to nucleic acids and permeabilizes bilayers. *Biochemistry* 36: 3008–3017.

**43** Javadpour, M.M., Juban, M.M., Lo, W.-C.J. et al. (1996). De novo antimicrobial peptides with low mammalian cell toxicity. *Journal of Medicinal Chemistry* 39: 3107–3113.

**44** Scheller, A., Oehlke, J., Wiesner, B. et al. (1999). Structural requirements for cellular uptake of **α**-helical amphipathic peptides. *Journal of Peptide Science* 5: 185–194.

**45** Yamashita, H., Kato, T., Oba, M. et al. (2016). Development of a cell-penetrating peptide that exhibits responsive change in its secondary structure in the cellular environment. *Scientific Reports* 6: 33003.

**46** Schmidt, S., Adjobo-Hermans, M.J.W., Kohze, R. et al. (2017). Identification of short hydrophobic cell-penetrating peptides for cytosolic peptide delivery by rational design. *Bioconjugate Chemistry* 28: 382–389.

**47** Akishiba, M., Takeuchi, T., Kawaguchi, Y. et al. (2017). Cytosolic antibody delivery by lipid-sensitive endosomolytic peptide. *Nature Chemistry* 9: 751–761.

**48** Liu, B.R., Huang, Y.-W., Aronstam, R.S. et al. (2016). Identification of α short cell-penetrating peptide from bovine lactoferricin for intracellular delivery of DNA in human A549 cells. *PLOS ONE* 11 (3): e0150439.

**49** Perrone, B., Miles, A.J., Salnikov, E.S. et al. (2014). Lipid interaction of LAH4, a peptide with antimicrobial and nucleic acid transfection activities. *European Biophysics Journal* 43: 499–507.

**50** Xu, Y., Liang, W., Qiu, Y. et al. (2016). Incorporation of a nuclear localization signal in pH responsive LAH4-L1 peptide enhances transfection and nuclear uptake of plasmid DNA. *Molecular Pharmaceutics* 13: 3141–3152.

**51** Zhang, Y., Li, L., Chang, L. et al. (2019). Design of a new pH-activatable cell-penetrating peptide for drug delivery into tumor cells. *Chemical Biology & Drug Design* 94: 1884–1893.

**52** Usui, K., Kikuchi, T., Mie, M. et al. (2013). Systematic screening of the cellular uptake of designed alpha-helix peptides. *Bioorganic & Medicinal Chemistry* 21: 2560–2567.

**53** Kozhikhova, K.V., Andreev, S.M., Shilovskiy, I.P. et al. (2018). A novel peptide dendrimer LTP efficiently facilitates transfection of mammalian cells. *Organic & Biomolecular Chemistry* 16: 8181–8190.

**54** Jiang, Y., Yang, W., Zhang, J. et al. (2018). Protein toxin chaperoned by LRP-1 targeted virus-mimicking vesicles induces high-efficiency glioblastoma therapy *in vivo*. *Advanced Materials* 30: 1800316.

**55** El-Andaloussi, S., Johansson, H.J., Holm, T. et al. (2007). A novel cell-penetrating peptide, M918, for efficient delivery of proteins and peptide nucleic acids. *Molecular Therapy* 15 (10): 1820–1826.

**56** Oehlke, J., Krause, E., Wiesner, B. et al. (1996). Nonendocytic, amphipathicity dependent cellular uptake of helical model peptides. *Protein and Peptide Letters* 3 (6): 393–398.

**57** Wada, S., Tsuda, H., Okada, T. et al. (2011). Cellular uptake of Aib-containing amphipathic helix peptide. *Bioorganic & Medicinal Chemistry Letters* 21: 5688–5691.

**58** Chen, J., Guan, S.-M., Sun, W. et al. (2016). Melittin, the major pain-producing substance of bee venom. *Neuroscience Bulletin* 32 (3): 265–272.

**59** Jones, S. and Howl, J. (2004). Charge delocalisation and the design of novel mastoparan analogues: enhanced cytotoxicity and secretory efficacy of [$Lys^5$, $Lys^8$, $Aib^{10}$]MP. *Regulatory Peptides* 121: 121–128.

**60** Morris, M.C., Vidal, P., Chaloin, L. et al. (1997). A new peptide vector for efficient delivery of oligonucleotides into mammalian cells. *Nucleic Acids Research* 25: 2730–2736.

**61** Lin, Y.-Z., Yao, S.Y., Veach, R.A. et al. (1995). Inhibition of nuclear translocation of transcription factor NF-κB by a synthetic peptide containing a cell membrane-permeable motif and nuclear localization sequence. *The Journal of Biological Chemistry* 270 (24): 14255–14258.

**62** Rojas, M., Donahue, J.P., Tan, Z. et al. (1998). Genetic engineering of proteins with cell membrane permeability. *Nature Biotechnology* 16: 370–375.

**63** Arukuusk, P., Parnaste, L., Oskolkov, N. et al. (2013). New generation of efficient peptide-based vectors, NickFects, for the delivery of nucleic acids. *Biochimica et Biophysica Acta* 1828: 1365–1373.

**64** Freimann, K., Arukuusk, P., Kurrikoff, K. et al. (2016). Optimization of *in vivo* DNA delivery with NickFect peptide vectors. *Journal of Controlled Release* 241: 135–143.

**65** Porosk, L., Arukuusk, P., Pohako, K. et al. (2019). Enhancement of siRNA transfection by the optimization of fatty acid length and histidine content in the CPP. *Biomaterials Science* 7: 4363–4374.

**66** Iwasaki, T., Tokuda, Y., Kotake, A. et al. (2015). Cellular uptake and *in vivo* distribution of polyhistidine peptides. *Journal of Controlled Release* 210: 115–124.

**67** Numata, K., Horii, Y., Oikawa, K. et al. (2018). Library screening of cell-penetrating peptide for BY-2 cells, leaves of Arabidopsis, tobacco, tomato, poplar, and rice callus. *Scientific Reports* 8: 10966.

**68** Douat, C., Aisenbrey, C., Antunes, S. et al. (2015). A cell-penetrating foldamer with a bioreducible linkage for intracellular delivery of DNA. *Angewandte Chemie* 127: 11285–11289.

**69** Taylor, B.N., Mehta, R.R., Yamada, T. et al. (2009). Noncationic peptides obtained from azurin preferentially enter cancer cells. *Cancer Research* 69 (2): 537–546.

**70** Jirka, S.M.G., Heemskerk, H., Winter, C.L.T. et al. (2014). Peptide conjugation of 2′-*O*-methyl phosphorothioate antisense oligonucleotides enhances cardiac uptake and exon skipping in *mdx* mice. *Nucleic Acid Therapeutics* 24 (1): 25–36.

**71** Kamei, N., Kikushi, S., Takeda-Morishita, M. et al. (2013). Determination of the optimal cell-penetrating peptide sequence for intestinal insulin delivery based on molecular orbital analysis with self-organizing maps. *Journal of Pharmaceutical Sciences* 102 (2): 469–479.

**72** Derossi, D., Joliot, A.H., Chassaing, G. et al. (1994). The third helix of the Antennapedia homeodomain translocates through biological membranes. *The Journal of Biological Chemistry* 269 (14): 10444–10450.

**73** Morris, M.C., Depollier, J., Mery, J. et al. (2001). A peptide carrier for the delivery of biologically active proteins into mammalian cells. *Nature Biotechnology* 19: 1173–1176.

**74** Gao, C., Mao, S., Ditzel, H.J. et al. (2002). A cell-penetrating peptide from a novel pVII-pIX phage-displayed random peptide library. *Bioorganic & Medicinal Chemistry* 10: 4057–4065.

**75** Marks, J.R., Placone, J., Hristova, K. et al. (2011). Spontaneous membrane-translocating peptides by orthogonal high-throughput screening. *Journal of the American Chemical Society* 133: 8995–9004.

**76** Ezzat, K., El Andaloussi, S., Zaghloul, E.M. et al. (2011). PepFect 14, a novel cell-penetrating peptide for oligonucleotide delivery in solution and as solid formulation. *Nucleic Acid Research* 39 (12): 5284–5298.

**77** Mae, M., El Andaloussi, S., Lundin, P. et al. (2009). A stearylated CPP for delivery of splice correcting oligonucleotides using a non-covalent co-incubation strategy. *Journal of Controlled Release* 134: 221–227.

**78** El Andaloussi, S., Lehto, T., Mager, I. et al. (2011). Design of a peptide-based vector, PepFect6, for efficient delivery of siRNA in cell culture and systemically *in vivo*. *Nucleic Acid Research* 39 (9): 3972–3987.

**79** Watkins, C.L., Brennan, P., Fegan, C. et al. (2009). Cellular uptake, distribution and cytotoxicity of the hydrophobic cell penetrating peptide sequence PFVYLI linked to the proapoptotic domain peptide PAD. *Journal of Controlled Release* 140: 237–244.

**80** Fahrner, R.L., Dieckmann, T., Harwig, S.S.L. et al. (1996). Solution structure of protegrin-1, a broad-spectrum antimicrobial peptide from porcine leukocytes. *Chemistry & Biology* 3: 543–550.

**81** Henry, K.E., Chaney, A.M., Nagle, V.L. et al. (2020). Demarcation of sepsis-induced peripheral and central acidosis with pH (low) insertion cycle peptide. *Journal of Nuclear Medicine* 61: 1361–1368.

**82** An, M., Wijesinghe, D., Andreev, O.A. et al. (2010). pH-(low)-insertion-peptide (pHLIP) translocation of membrane impermeable phalloidin toxin inhibits cancer cell proliferation. *Proceedings of the National Academy of Sciences of the United States of America* 107: 20246–20250.

**83** Lehto, T., Alvarez, A.C., Gauck, S. et al. (2014). Cellular trafficking determines the exon skipping activity of Pip6a-PMO in *mdx* skeletal and cardiac muscle cells. *Nucleic Acid Research* 42 (5): 3207–3217.

**84** Rittner, K., Benavente, A., Bompard-Sorlet, A. et al. (2002). New basic membrane-destabilizing peptides for plasmid-based gene delivery *in vitro* and *in vivo*. *Molecular Therapy* 5 (2): 104–114.

**85** Kanekura, K., Harada, Y., Fujimoto, M. et al. (2018). Characterization of membrane penetration and cytotoxicity of C9orf72-encoding arginine-rich dipeptides. *Scientific Reports* 8: 12740.

**86** Oess, S. and Hildt, E. (2000). Novel cell permeable motif derived from the PreS2-domain of hepatitis-B virus surface antigens. *Gene Therapy* 7: 750–758.

**87** Ho, A., Schwarze, S.R., Mermelstein, S.J. et al. (2001). Synthetic protein transduction domains: enhanced transduction potential *in vitro* and *in vivo*. *Cancer Research* 61: 474–477.

**88** Elmquist, A., Lindgren, M., Bartfai, T. et al. (2001). VE-cadherin-derived cell-penetrating peptide, *p*VEC, with carrier functions. *Experimental Cell Research* 269: 237–244.

**89** Delaroche, D., Aussedat, B., Aubry, S. et al. (2007). Tracking a new cell-penetrating (W/R) nonapeptide, through an enzyme-stable mass spectrometry reporter tag. *Analytical Chemistry* 79: 1932–1938.

**90** Moulton, H.M., Nelson, M.H., Hatlevig, S.A. et al. (2004). Cellular uptake of antisense morpholino oligomers conjugated to arginine-rich peptides. *Bioconjugate Chemistry* 15: 290–299.

**91** Gao, L., Yu, J., Liu, Y. et al. (2018). Tumor-penetrating peptide conjugated and doxorubicin loaded $T_1$-$T_2$ dual mode MRI contrast agents nanoparticles for tumor theranostics. *Theranostics* 8 (1): 92–108.

**92** Vaissiere, A., Aldrian, G., Konate, K. et al. (2017). A *retro-inverso* cell-penetrating peptide for siRNA delivery. *Journal of Nanobiotechnology* 15: 34.

**93** Nakase, I., Okumura, S., Katayama, S. et al. (2012). Transformation of an antimicrobial peptide into a plasma membrane-permeable, mitochondria-targeted peptide *via* the substitution of lysine with arginine. *Chemical Communications* 48: 11097–11099.

**94** Oba, M., Ito, Y., Umeno, T. et al. (2019). Plasmid DNA delivery using cell-penetrating peptide foldamers composed of Arg-Arg-Aib repeating sequences. *ACS Biomaterials Science & Engineering* 5: 5660–5668.

**95** Oba, M., Nagano, Y., Kato, T. et al. (2019). Secondary structures and cell-penetrating abilities of arginine-rich peptide foldamers. *Scientific Reports* 9: 1349.

**96** Jobin, M.-L., Vamparys, L., Deniau, R. et al. (2019). Biophysical insight on the membrane insertion of an arginine-rich cell-penetrating peptide. *International Journal of Molecular Sciences* 20: 4441.

**97** Safa, N., Anderson, J.C., Vaithiyanathan, M. et al. (2019). CPProtectides: rapid uptake of well-folded β-hairpin peptides with enhanced resistance to intracellular degradation. *Peptide Science* 111: e24092.

**98** Pinto, A., Lennarz, S., Rodrigues-Correia, R. et al. (2012). Functional detection of proteins by caged aptamers. *ACS Chemical Biology* 7: 360–366.

**99** Pujals, S. and Giralt, E. (2008). Proline-rich, amphipathic cell-penetrating peptides. *Advanced Drug Delivery Review* 60: 473–484.

**100** Martin, I., Teixido, M., and Giralt, E. (2011). Design, synthesis and characterization of a new anionic cell-penetrating peptide: SAP(E). *ChemBioChem* 12: 896–903.

**101** Hoyer, J., Schatzschneider, U., Schulz-Siegmund, M. et al. (2012). Dimerization of a cell-penetrating peptide leads to enhanced cellular uptake and drug delivery. *Beilstein Journal of Organic Chemistry* 8: 1788–1797.

**102** Mello, L.R., Aguiar, R.B., Yamada, R.Y. et al. (2020). Amphipathic design dictates self-assembly, cytotoxicity and cell uptake of arginine-rich surfactant-like peptides. *Journal of Material Chemistry B* 8: 2495–2507.

**103** Lamaziere, A., Burlina, F., Wolf, C. et al. (2007). Non-metabolic membrane tubulation and permeability induced by bioactive peptides. *PLOS ONE* 2 (2): e201.

**104** Futaki, S., Ohashi, W., Suzuki, T. et al. (2001). Stearylated arginine-rich peptides: a new class of transfection systems. *Bioconjugate Chemistry* 12: 1005–1011.

**105** Khalil, I.A., Kogure, K., Futaki, S. et al. (2006). High density of octaarginine stimulates micropinocytosis leading to efficient intracellular trafficking for gene expression. *The Journal of Biological Chemistry* 281 (6): 3544–3551.

**106** Hilinski, G.J., Kim, Y.-W., Hong, J. et al. (2014). Stitched α-helical peptides via bis ring-closing metathesis. *Journal of the American Chemical Society* 136: 12314–12322.

**107** Stinthvanich, C., Veiga, A.S., Gupta, K. et al. (2012). Anticancer β-hairpin peptides: membrane-induced folding triggers activity. *Journal of the American Chemical Society* 134: 6210–6217.

**108** Rousselle, C., Clair, P., Lefauconnier, J.-M. et al. (2000). New advances in the transport of doxorubicin through the blood-brain barrier by a peptide vector-mediated strategy. *Molecular Pharmacology* 57 (4): 679–686.

**109** Jain, A., Yadav, B.K., and Chugh, A. (2015). Marine antimicrobial peptide tachyplesin as an efficient nanocarrier for macromolecule delivery in plant and mammalian cells. *The FEBS Journal* 282: 732–745.

**110** Vives, E., Brodin, P., and Lebleu, B. (1997). A truncated HIV-1 Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus. *The Journal of Biological Chemistry* 272: 16010–16017.

**111** Zhang, W., Song, J., Zhang, B. et al. (2011). Design of acid-activated cell penetrating peptide for delivery of active molecules into cancer cells. *Bioconjugate Chemistry* 22: 1410–1415.

**112** Song, J., Kai, M., Zhang, W. et al. (2011). Cellular uptake of transportan 10 and its analogs in live cells: selectivity and structure-activity relationship studies. *Peptides* 21: 1934–1941.

**113** Bleifuss, E., Kammertoens, T., Hutloff, A. et al. (2006). The translocation motif of hepatitis B virus improves protein vaccination. *Cellular and Molecular Life Sciences* 63: 627–635.

**114** Soomets, U., Lindgren, M., Gallet, X. et al. (2000). Deletion analogues of transportan. *Biochimica et Biophysica Acta* 1467: 165–176.

**115** Pooga, M., Hallbrink, M., Zorko, M. et al. (1998). Cell penetration of transportan. *The FASEB Journal* 12: 67–77.

**116** Elliott, G. and O'Hare, P. (1997). Intercellular trafficking and protein delivery by a herpesvirus structural protein. *Cell* 88: 223–233.

**117** Gross, D.A., Leborgne, C., Chappert, P. et al. (2019). Induction of tumor-specific CTL responses using the C-terminal fragment of Viral protein R as cell penetrating peptide. *Scientific Reports* 9: 3937.

**118** Oehlke, J., Krause, E., Wiesner, B. et al. (1997). Extensive cellular uptake into endothelial cells of an amphipathic β-sheet forming peptide. *FEBS Letters* 415: 196–199.

**119** Konate, K., Dussot, M., Aldrian, G. et al. (2019). Peptide-based nanoparticles to rapidly and efficiently "Wrap 'n Roll" siRNA into cells. *Bioconjugate Chemistry* 30: 592–603.