

CPP, Cell-Penetrating Peptides

Ülo Langel

CPP, Cell-Penetrating Peptides

 Springer

Ülo Langel
Department of Biochemistry and Biophysics
Stockholm University
Stockholm, Stockholms Län, Sweden

Institute of Technology
University of Tartu
Tartu, Estonia

ISBN 978-981-13-8746-3 ISBN 978-981-13-8747-0 (eBook)
<https://doi.org/10.1007/978-981-13-8747-0>

© Springer Nature Singapore Pte Ltd. 2019

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd. The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Contents

| | | |
|----------|---|----|
| 1 | Introduction | 1 |
| | References | 12 |
| 2 | Classes and Applications of Cell-Penetrating Peptides | 29 |
| 2.1 | Protein Derived Versus Designed | 29 |
| 2.2 | Classification by Physico-chemical Properties Versus Structural Properties | 32 |
| 2.3 | Predicted Versus Random | 34 |
| 2.4 | Linear Versus Modified | 35 |
| 2.4.1 | Linear | 35 |
| 2.4.2 | Stabilized | 39 |
| 2.5 | Protein Mimics Versus Cargo Delivery Vectors | 44 |
| 2.5.1 | Protein-Mimicking CPPs | 44 |
| 2.5.2 | CPPs for Cargo Delivery | 46 |
| 2.6 | Nonspecific Versus Targeted | 51 |
| 2.6.1 | Affinity Targeting: Homing, Ligand Based Targeting | 52 |
| 2.6.2 | Prodrug Approach, ACPP | 55 |
| 2.7 | Classification by Uptake Mechanisms | 56 |
| 2.7.1 | Direct Translocators | 56 |
| 2.7.2 | Endocytosis Enhancers | 57 |
| 2.8 | Non-toxic Versus Antimicrobial | 60 |
| 2.8.1 | Non-toxic | 61 |
| 2.8.2 | Antimicrobial | 61 |
| | References | 63 |
| 3 | Methods for CPP Functionalization | 83 |
| 3.1 | Selection, Prediction and in Silico Analysis | 84 |
| 3.2 | Labeling | 88 |
| 3.2.1 | Radioactive CPP Labelling | 90 |
| 3.2.2 | Fluorescent Labelling of CPPs | 91 |

| | | |
|----------|--|------------|
| 3.2.3 | Quantum Dots | 95 |
| 3.2.4 | Fluorescence Quenching | 97 |
| 3.3 | Functionalization of CPPs | 100 |
| 3.3.1 | Luciferin-CPP | 100 |
| 3.3.2 | Antisense | 101 |
| 3.3.3 | siRNA Delivery Methods | 111 |
| 3.3.4 | Additional Gene Therapeutic Platforms | 119 |
| | References | 134 |
| 4 | Protein Delivery and Mimicry | 157 |
| 4.1 | Fusion Proteins | 158 |
| 4.2 | Chemical Conjugation of CPPs to Proteins | 161 |
| 4.3 | Biotin Conjugations | 162 |
| 4.4 | Complexation of Proteins and Peptides | 164 |
| 4.5 | Targeting Proteins with CPPs | 166 |
| 4.6 | Protein Mimicry and PPI | 170 |
| 4.6.1 | Delivery of Protein Mimics as Cargos by CPPs | 171 |
| 4.6.2 | Protein Mimics with CPP Properties | 175 |
| | References | 179 |
| 5 | Targeting Strategies | 195 |
| 5.1 | “Addressing” | 195 |
| 5.2 | Prodrug Strategies | 201 |
| 5.3 | Targeting Intracellular Organelles with CPPs | 206 |
| 5.4 | Targeting Biological Tissue Barriers | 216 |
| 5.5 | Targeting Plants | 230 |
| | References | 234 |
| 6 | Methods for Detection and Visualization of CPPs | 265 |
| 6.1 | Fluorescence Activated Cell Sorting, FACS | 270 |
| 6.2 | Fluorescence Correlation Spectroscopy, FCS | 271 |
| 6.3 | HPLC Analysis | 271 |
| 6.4 | Mass-Spectrometric Methods | 272 |
| 6.5 | Fluorescence Quenching | 274 |
| 6.6 | Electron Microscopy | 276 |
| | References | 279 |
| 7 | Methods for Structural Studies of CPPs | 289 |
| 7.1 | Model Membranes | 289 |
| 7.2 | Circular Dichroism, CD | 298 |
| 7.3 | Nuclear Magnetic Spectroscopy, NMR | 303 |
| 7.4 | Dynamic Light Scattering (DLS) | 308 |
| | References | 310 |
| 8 | Kinetics of CPPs Cellular Uptake | 325 |
| 8.1 | Kinetics by Stimulations | 327 |
| 8.2 | Micelle Kinetics | 327 |

| | | |
|-----------|---|------------|
| 8.3 | Kinetics in Cells | 328 |
| 8.4 | Kinetics and Mechanisms | 331 |
| | References | 334 |
| 9 | Toxicity and Immune Response | 339 |
| 9.1 | Basic Methods for Determination of Cytotoxicity and Immunogenic Activities of CPPs | 339 |
| 9.2 | Toxicity Issues of CPPs | 341 |
| 9.3 | Immune Response and CPPs | 344 |
| | References | 349 |
| 10 | Cell-Translocation Mechanisms of CPPs | 359 |
| 10.1 | Membrane Interactions, Mechanisms of Direct Penetration | 361 |
| 10.1.1 | Examples of CPP Direct Translocation | 361 |
| 10.1.2 | Mechanisms of Direct Translocation | 362 |
| 10.2 | Endocytotic Uptake | 365 |
| 10.2.1 | Endocytotic CPP Uptake and Involvement of Cell Surface Receptors | 365 |
| 10.2.2 | Binding Affinity of CPP Interactions | 368 |
| 10.2.3 | Mechanisms of Endosomal Escape | 372 |
| 10.3 | Signaling in CPP Internalization | 375 |
| 10.3.1 | Proteomic, Genomic, Transcriptomic, Metabolomic Studies | 379 |
| | References | 381 |
| 11 | Clinical Trials and Commercialization Using CPPs | 395 |
| 11.1 | Protein Mimics and Other Peptides | 395 |
| 11.2 | New Modalities | 400 |
| 11.3 | Antisense Strategies | 403 |
| | References | 404 |
| 12 | Therapeutic Potential of CPPs | 409 |
| 12.1 | Antimicrobial and Antiviral Applications | 411 |
| 12.2 | Potential in Cancer Therapy | 415 |
| 12.3 | Cardiac Diseases | 423 |
| 12.4 | Duchenne Muscular Dystrophy | 424 |
| 12.5 | Transplant Rejection | 425 |
| 12.6 | Addressing Blood-Brain-Barrier | 426 |
| 12.7 | Molecular Imaging In Vivo | 430 |
| 12.8 | Concluding Remarks | 433 |
| | References | 436 |
| | Index | 463 |

Abbreviations

| | |
|----------|---|
| Aa | Amino acid |
| Abz | 2-Aminobenzoic acid |
| aCPP | Activatable cell-penetrating peptide |
| AD | Alzheimer's disease |
| Aib | α -Aminoisobutyric acid |
| ALA | 5-Aminolevulinic acid |
| AMP | Antimicrobial peptides |
| APP | Amyloid precursor protein |
| ASO | Antisense oligonucleotide |
| ATTEMPTS | Antibody targeted triggered electrically modified prodrug type strategy |
| BBB | Blood-brain-barrier |
| BK | Bradykinin |
| bPrPp | Bovine prion protein |
| CaM | Calmodulin |
| Cas | CRISPR-associated protein systems |
| CatD | Cathepsin D |
| CD | Circular dichroism |
| CF | Carboxyfluorescein |
| Chol | Cholesteryl |
| CLSM | Confocal laser scanning microscopy |
| CNS | Central nervous system |
| CPP | Cell-penetrating peptide |
| CRISPR | Clustered regularly interspaced short palindromic repeat |
| DDS | Drug delivery systems |
| DLS | Dynamic light scattering |
| DMD | Duchenne Muscular Dystrophy |
| DOX | Doxorubicin |
| DPC | Dodecylphosphocholine |
| DRBD | Double-stranded RNA-binding domain |

| | |
|-------|---|
| EED | Endosomal escape domains |
| EGFR | Epidermal growth factor receptor |
| EM | Electron microscopy |
| EPR | Enhanced permeation and retention |
| ERT | Enzyme replacement therapy |
| FA | Folic acid |
| FAM | Arboxyfluorescein |
| FACS | Fluorescence activated cell sorting |
| FCS | Fluorescence correlation spectroscopy |
| FITC | Fluorescein isothiocyanate |
| Fl | Fluorescent label |
| FRET | Förster resonance energy transfer |
| GAGs | Glycosaminoglycans |
| GET | Glycosaminoglycan-binding enhanced transduction delivery system |
| GFP | Green fluorescent protein |
| GO | Graphene oxide |
| GPCR | G-protein coupled receptor |
| GUV | Giant unilamellar vesicles |
| HA | Haemagglutinin |
| hMSC | Human mesenchymal stem cell |
| HPLC | High performance liquid chromatography |
| HS | Heparan sulfate |
| HSC | Haematopoietic stem cell |
| Hsp70 | Heat shock protein 70 |
| HSPG | Heparan sulfate proteoglycan |
| IgG | Immunoglobulin G |
| iPS | Induced pluripotent stem cell |
| iRGD | Integrin-binding RGD motif |
| ITC | Isothermal titration calorimetry |
| JIP | JNK interacting protein |
| LF | Lactoferrin |
| LMWP | Low molecular weight protamine |
| LNA | Locked nucleic acid |
| LSP | Lysosomal sorting peptide |
| LUV | Large unilamellar vesicles |
| mAb | Monoclonal antibody |
| MAP | Membrane active peptide |
| MD | Molecular dynamics |
| MEND | Multifunctional envelope-type nanodevice |
| MHC | Major histocompatibility complex |
| MION | Magnetic iron oxide nanoparticles |
| miRNA | MicroRNA |
| MLV | Multilamellar vesicles |
| MMP | Matrix metalloprotease |
| MPP | Mitochondria-penetrating peptides |

| | |
|-------|---|
| MRI | Magnetic resonance imaging |
| MS | Mass-spectrometry |
| MSC | Mesenchymal stem cells |
| mt | Mitochondria |
| MTS | Mitochondrial targeting sequence |
| NLC | Nanostructured lipid carrier |
| NLS | Nuclear localisation signal |
| NMR | Nuclear magnetic resonance |
| NP | Nanoparticle |
| NRP-1 | Neuropilin-1 |
| ON | Oligonucleotide |
| OVA | Ovalbumin |
| PACAP | Pituitary adenylate-cyclase-activating polypeptide |
| pAntp | <i>Drosophila</i> homeoprotein Antennapedia derived peptide |
| pArg | Poly-arginine, pR |
| PC | Phosphatidylcholine |
| PCM | Primary cardiomyocyte-targeting peptide |
| pDNA | Plasmid DNA |
| PDT | Photodynamic therapy |
| PDZ | A 80–90 aa domain found in the signaling proteins |
| PEG | Poly-ethylene glycol |
| PEI | Polyethylenimine |
| Pen | Penetratin |
| PET | Positron-emission tomography |
| PF | PepFect |
| pHLIP | pH low insertion peptides |
| Pip | PNA internalising peptide |
| PK | Protein kinase |
| PLA | Poly-l-arginine |
| PLGA | Poly(dl-lactic acid-co-glycolic acid) |
| PLL | Poly-l-lysine (PLL) |
| PMO | Phosphorodiamidate morpholino oligomers |
| PNA | Peptide nucleic acid |
| POPC | 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine |
| POPG | 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol |
| PPI | Protein-protein interaction |
| PS | Phosphorothioate ON |
| PSC | Pluripotent stem cells |
| PSP | Photo-sensitive peptide |
| PTD | Protein/peptide transduction domains |
| PTX | Paclitaxel (PTX) |
| QD | Quantum dots |
| R8 | Octa-arginine |
| Rh | Rhodamine |
| ri | Retro-inverso |

| | |
|--------|---|
| Rn | Poly-arginine |
| SAP | Sweet arrow peptide |
| SAR | Structure-activity studies |
| SCARA | Class A scavenger receptors |
| SCO | Splice correcting ON |
| scFv | Single chain variable fragment |
| SDS | Sodium dodecyl sulfate |
| SEM | Scanning electron microscope |
| siRNA | Short interfering RNA |
| SOD | Suoeroxide dismutase |
| SPPS | Solid phase peptide synthesis |
| SPT | Single particle tracking |
| SUV | Small unilamellar vesicles |
| TALENs | Transcription activator like effector nucleases |
| Tat | HIV-Tat derived CPP |
| TEM | Transmission electron microscopy |
| Tf | Transferrin |
| TF | Transcription factor |
| THP | Tumour homing peptide |
| TP | Transportan |
| TPP | Tumor penetrating peptide |
| TRITC | Tetramethyl rhodamine iso-thiocyanate |
| Yops | Human-pathogenic Yersinia plasmid-encoded Yersinia outer proteins |
| ZFNs | Zinc finger nucleases |