evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: Synthesis of peptides, their conjugation, labelling and use for cancer cell penetration and drug delivery

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Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik

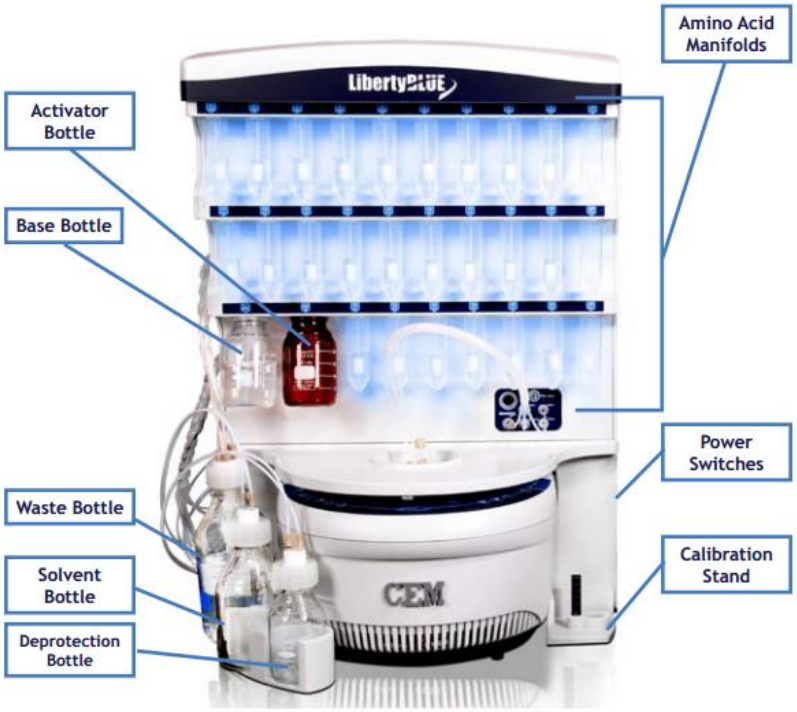
Cell penetrating peptide – history of synthesis

- 1965 - Ryser, Hancock – first description of polycationic peptide capable of traversing the cellular plasma membrane
- 1988 - Lebleu's - first CPP trans-activating transcriptional activator (Tat)
- 1994 – Prochiantz - cell-penetrating properties of penetratin

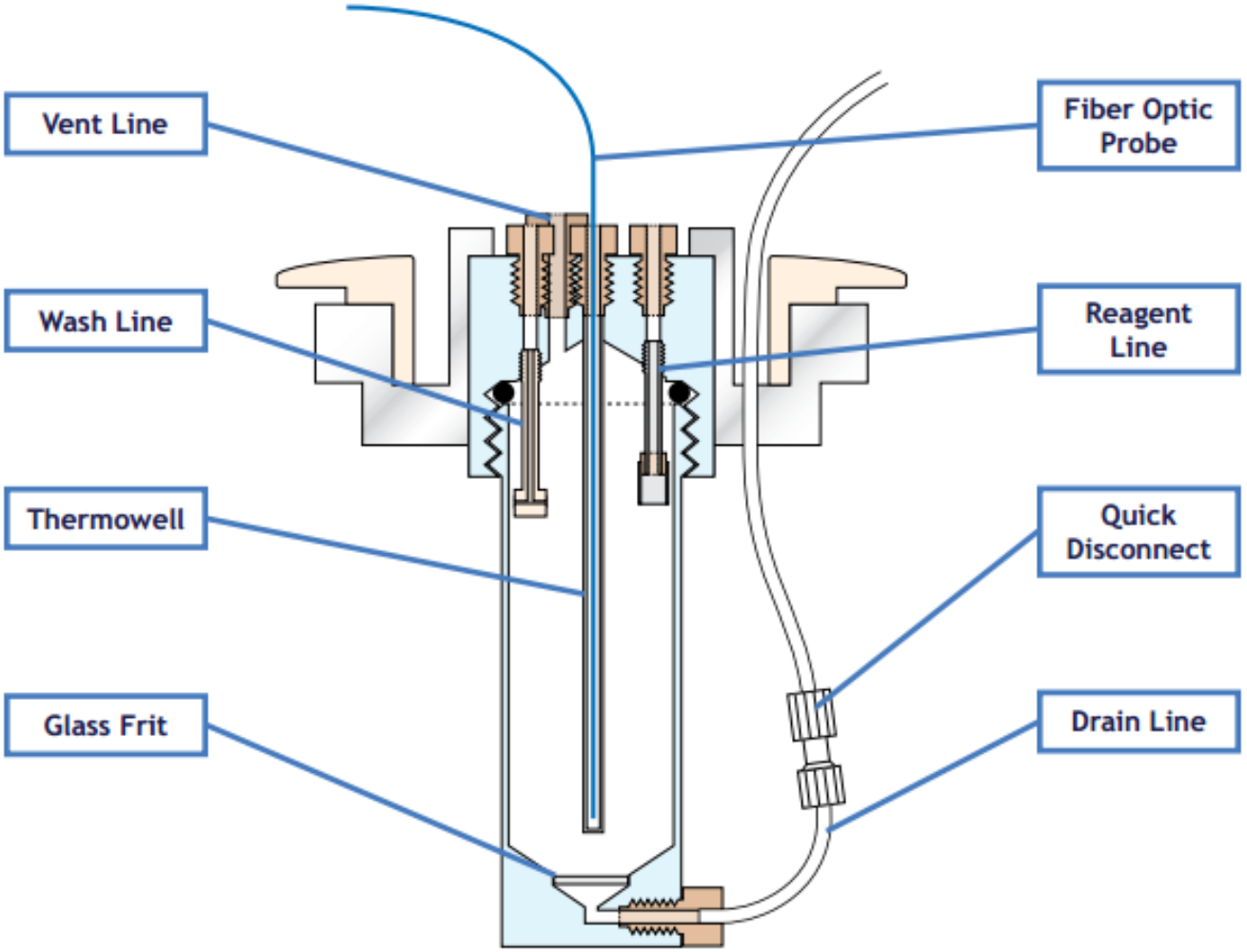
Characteristics

- (CPPs) are short cationic and/or amphipathic peptides, usually less than 30 amino acids
- CPPs can be arranged in three classes: protein derived CPPs, model peptides, and designed CPPs.
- Conjugation with different macromolecules (proteins, nucleic acids, siRNA, peptide nucleic acids (PNAs), small molecule therapeutics and quantum dots)
- Delivering cargo into cells (endocytosis or direct penetration)

Peptide Synthesizer

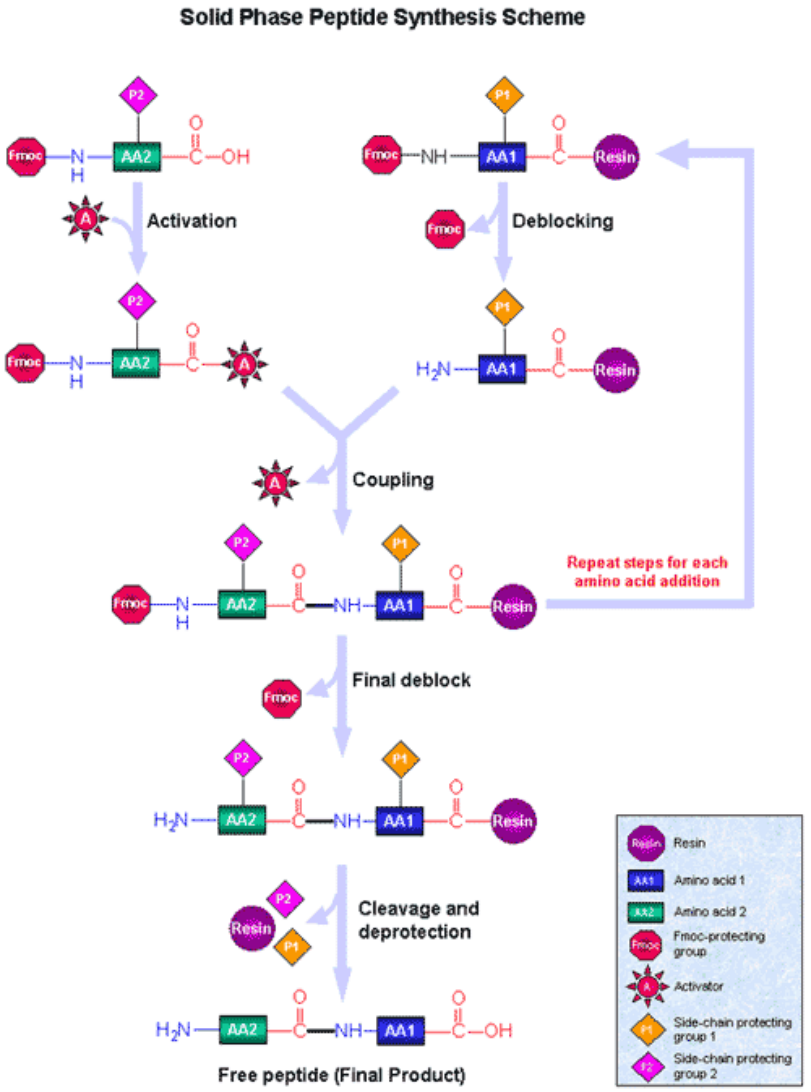


Reaction Vessel



Solid phase peptide synthesis

- Resin swelling
- Amino acid
- Deprotection
- Washing
- Microwave coupling
- Final deprotection
- Cleavage
- Isolation of peptide
- Lyophilization
- Maldi
- HPLC



Reagents for peptide synthesis

Activator and activator base

CEM Preference	Reagents	Cycle	Exceptions
1	AA/DIC/Oxyma in DMF	Single Amino Acid	His: Use 50 °C Cycle Arg: Use Double Arg Cycle
2	AA/HBTU/DIEA in DMF	Single Amino Acid	Cys: Use 50 °C Cycle His: Use 50 °C Cycle Arg: Use Double Arg Cycle
3	AA/DIC/Oxyma in NMP	Modified Single Amino Acid	Cys: Use 50 °C Cycle His: Use 50 °C Cycle Arg: Use Double Arg Cycle
4	AA/HBTU/DIEA in NMP	Modified Single Amino Acid	His: Use 50 °C Cycle Arg: Use Double Arg Cycle

Deprotection

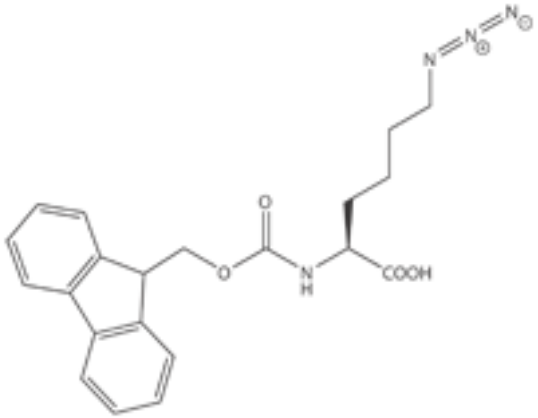
CEM Preference	Deprotection Cocktail
1	10% (w/v) Piperazine in 10:90 (EtOH:NMP)
2	20% Piperidine (v/v) in DMF or NMP

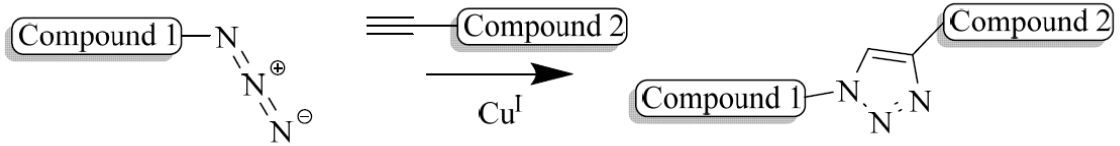
Cleavage cocktails

	Recipe	Hrs	Comments
B	TFA/phenol/water/ TIPS (88/5/5/2)	1-4	All peptides[1].
K	TFA/phenol/water/ thioanisole/EDT (82.5/5/5/5/2.5)	1-4	All peptides[1].
K'	TFA/phenol/water/ thioanisole/ 1-dodecanethiol (82.5/5/5/5/2.5)	1-4	All peptides[1].
L	TFA/DTT/Water/ TIPS (88/5/5/2)	1-4	All peptides[1]
P	TFA/phenol (95/5)	1-4	*t*Bu group. Do not use with Trp, Met or Cys.
P+	TFA/phenol/ Methanesulfonic acid (95/2.5/2.5)	15 min	All peptides[1,2].
R	TFA/thioanisole/ EDT/anisole (90/5/3/2)	1-8	All peptides[1].
T	TFA/TES (95/5)	1-4	Boc, *t*Bu,Trt. Do not use with Arg or Trp.
	TFA/water (95/5)	1-4	Boc, *t*Bu, Trt, Pbf[3]. Do not use with Trp, Met or Cys.
	TFA/DCM/indole (70/28/2)	1-4	Do not use with Arg[1].

Conjugation of peptide

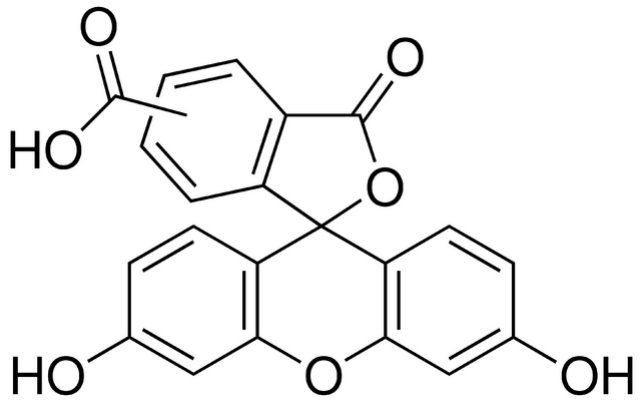
- Poor cellular uptake – charge neutral oligonucleotide, labelling peptide, peptide nucleic acids (PNA) or phosphorodiamidate morpholino oligomers (PMO), therapeutic drugs
- CPPs (Tat or Penetratin)
- Peptide linkers – e-Azidocaproic acid, Fmoc-azidolysine
- Modification of peptide – Azid and Alkyn group
- Peptide Conjugation via 'Click' Chemistry





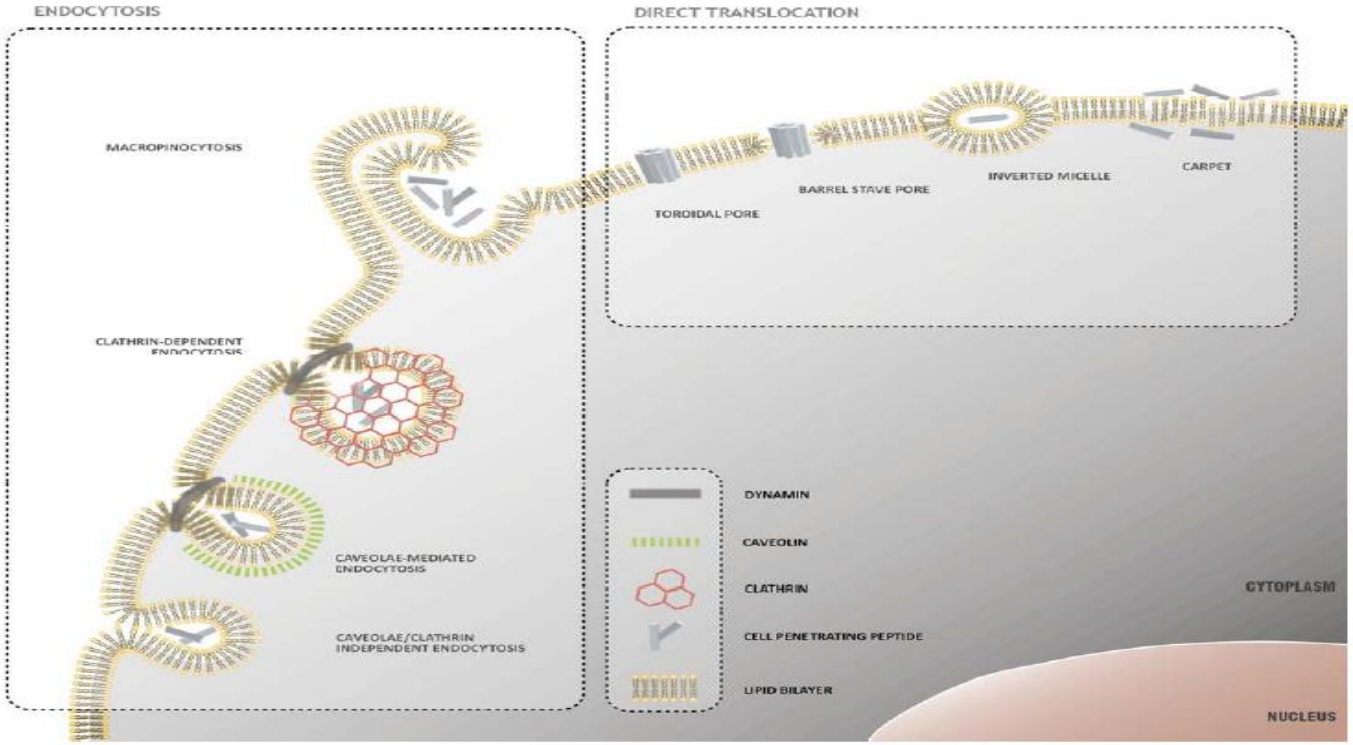
Labelling Peptides

- Dye-labeled fluorescent peptides -important tools for cellular studies
- Used in fluorescence microscopy, fluorescence fluorimetry, fluorescence resonance energy transfer (FRET)
- High fluorescence quantum yields
- Stable and not destructive under most biological condition
- 5(6)-Carboxyfluorescein



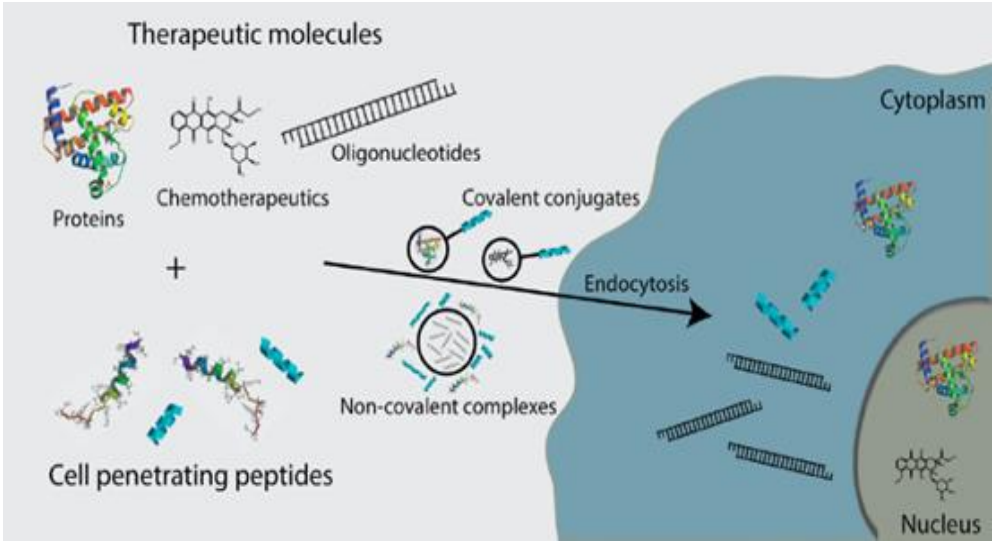
Anticancer activities of CPPs peptides

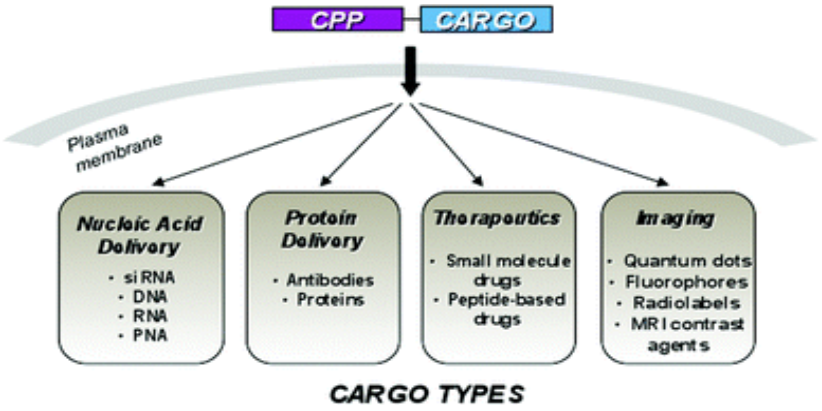
- High specificity against cancer cells - destroying cancer cells and protecting normal cells
- CPPs hydrophobicity and electrostatic interaction
- Effects of anticancer peptides - cytoplasmic membrane disruption via micellization or pore formation, and induction of apoptosis



- Cancer cells develop resistance to these drugs - anticancer peptides alternative chemotherapeutic agents

- Delivering biomolecules - antigenic peptides, peptide nucleic acids, antisense oligonucleotides, full-length proteins, or even and liposomes





Future of CPPs

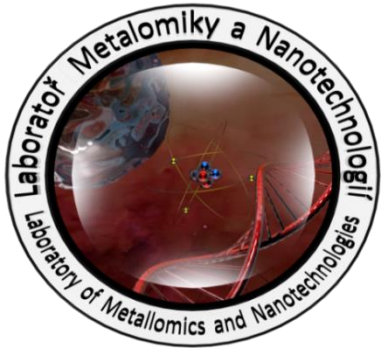
- Killing cancer cells while protecting normal cells and helping patients to recover rapidly
- Conjugation of therapeutic agents to CPPs
- Cancer cells develop resistance to these drugs - CPPs as alternative
- Selective cytotoxicity
- No restriction with respect to the size or type of cargo
- CPPs drug constructs have great potential to increase the solubility, biodistribution, and pharmacokinetic profiles of currently approved chemotherapeutic drugs.

Antitumor activity of buforin IIb against 60 human tumor cell lines*

Cell lines	IC_{50} (μg/ml)	Cell lines	IC_{50} (μg/ml)	Cell lines	IC_{50} (μg/ml)
Leukemia		CNS cancer		Ovarian cancer	
CCRF-CEM	14.7	SF-268	12.4	IGROV1	9.0
HL-60	11.3	SF-295	12.9	OVRCAR-3	15.2
K-562	8.2	SF-539	9.5	OVRCAR-4	17.6
MOLT-4	17.0	SNB-19	13.8	OVRCAR-5	13.8
RPMI-8226	10.5	SNB-75	15.5	OVRCAR-8	13.0
SR	20.2	U251	10.6	SK-OV-3	12.6
Breast cancer		Melanoma		Colon cancer	
MCF7	15.1	LOX IMVI	9.5	COLO 205	11.2
NCI/ADR-RES	11.5	MALME-3M	10.9	HCT-116	14.6
MDA-MB-231	11.3	M14	15.1	HCT-15	13.2
HS 578T	11.7	SK-MEL-2	11.1	HT29	17.6
MDA-MB-435	11.3	SK-MEL-5	8.9	KM12	12.0
MDA-N	10.6	UACC-257	12.5	SW-620	12.7
BT-549	12.9	UACC-62	10.6		
T-47D	23.9				
Non-small cell lung cancer		Renal cancer		Prostate cancer	
A549	11.7	786-0	12.2	PC-3	12.0
EKVX	12.1	A498	10.0	DU-145	15.3
HOP-62	12.6	ACHN	12.0		
HOP-92	7.2	CAKI-1	14.1		
NCI-H226	13.3	RFX 393	11.0		
NCI-H23	10.8	SN12C	11.4		
NCI-H322M	10.7	TK-10	13.1		
NCI-H460	12.1	UO-31	10.6		
NCI-H522	11.2				

Examples of cell-penetrating peptides (CPPs)

Name sequence	**Class** source
Penetratin	**protein derived CPP**
RQIKIWFQNRRMKWKK[a]	*Drosophila* Antennapedia homeodomain (amino acids 43–58)
Tat	**protein derived CPP**
CGRKKRRQRRRPPQC[a]	protein from human immunodeficiency virus 1 (amino acids 48–60)
pVEC	**protein derived CPP**
LLIILRRRIRKQAHAHSK-amide	derived from murine vascular endothelial cadherin
MAP	**model peptide**
KLALKLALKALKAALKLA-amide	
$(Arg)_7$	**model peptide**
RRRRRRR	
MPG	**designed CPP**
GALFLGFLGAAGSTMGAWSQPKSKRKV	peptide derived from fusion sequence of HIV-1 gp41 protein coupled to peptide derived from the nuclear localization sequence of SV40 T-antigen
Transportan	**designed CPP**
GWTLNSAGYLLGKINLKALAALAKISIL-amide	minimal active part of galanin (amino acids l–12) coupled to mastoparan via Lys^{13}



Mendel University in Brno

Thank you for your attention!

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