Present state of membrane permeable peptides and its application

Literature seminar (2018. 1. 20)
M1　Hiroki Horigome

Contents

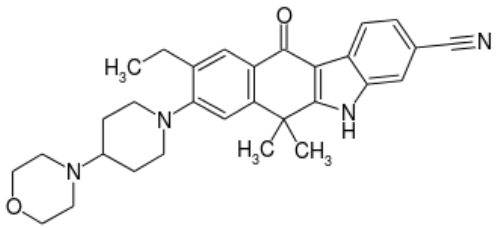
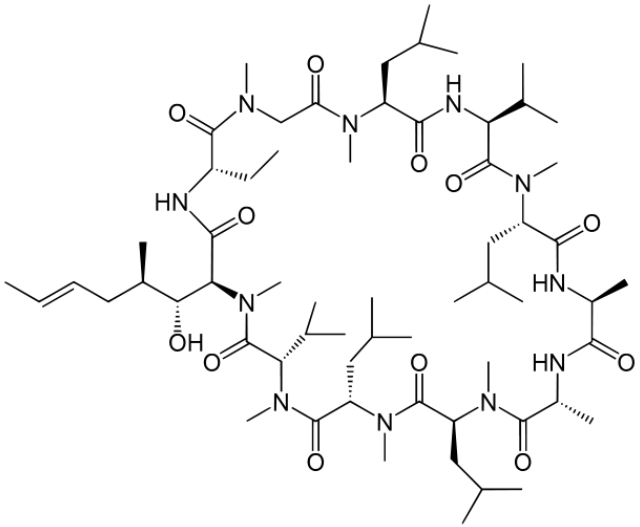
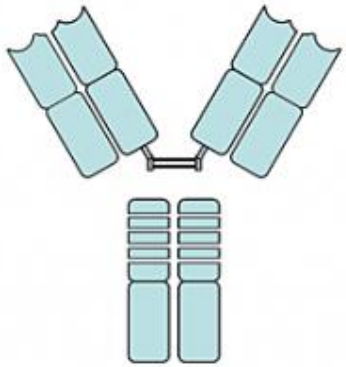
1. Current status of pharmaceutical products and cell permeable peptides.

2. Classification of Cell-Penetrating Peptides (CPPs)
   2-1. Cationic CPPs
   2-2. Amphipathic CPPs
   2-3. Hydrophobic CPPs

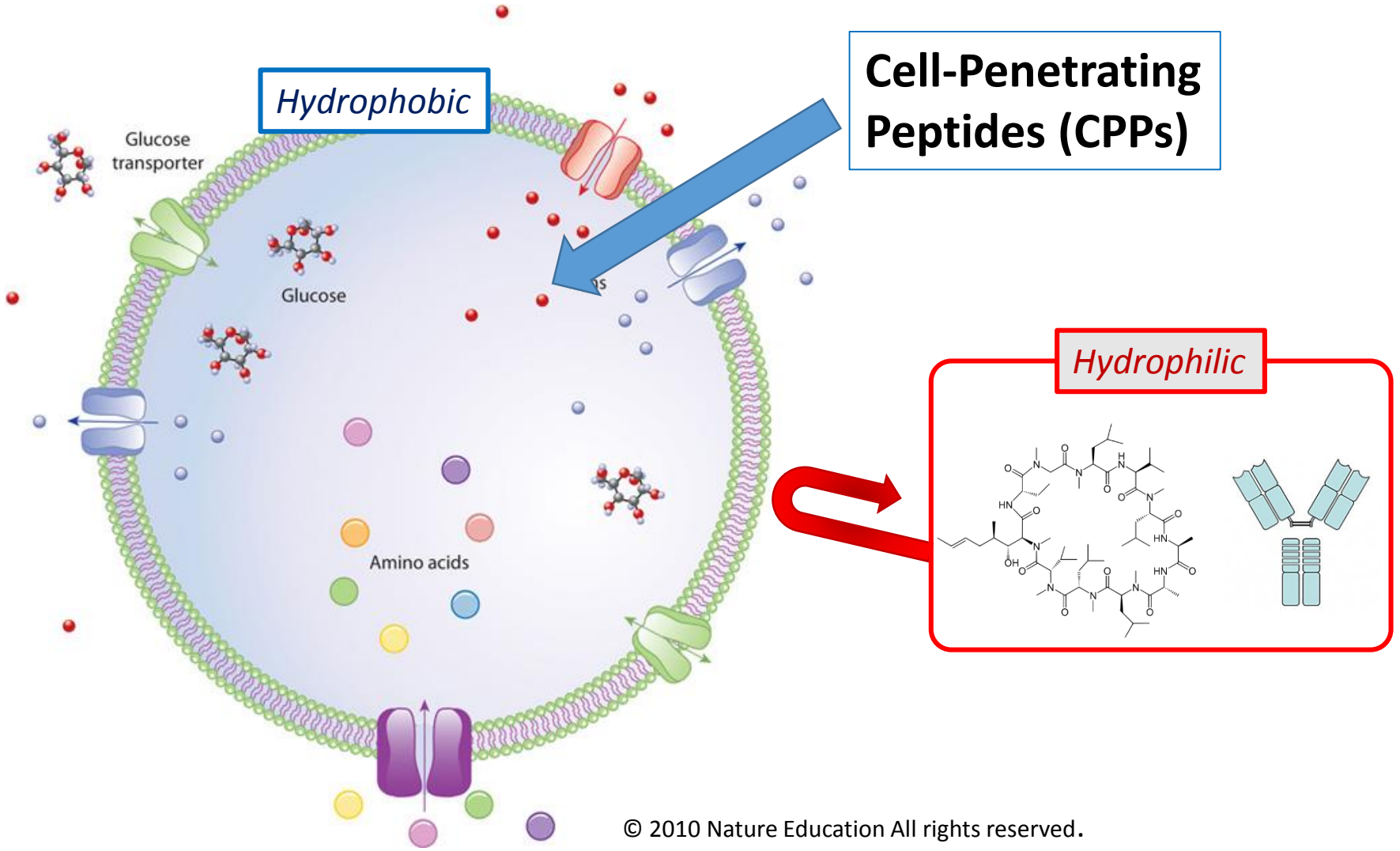
3. Applications of CPPs

1. **Current status of pharmaceutical products and cell permeable peptides.**

Transition of pharmaceuticals to larger compounds

	Small molecule	Medium molecule	Antibody drugs
Structure	 ex. Alectinib	 ex. Ciclosporin	
Molecular weight	**500 <**	**500 ～ 2000**	**≒ 150000**
Specificity	**Low**	***High***	***High***
Side effect	**High**	***Low***	***Low***

Cell membrane : barrier function



Specialized proteins in the cell membrane regulate the concentration of specific molecules inside the cell.

HIV-1 TAT protein

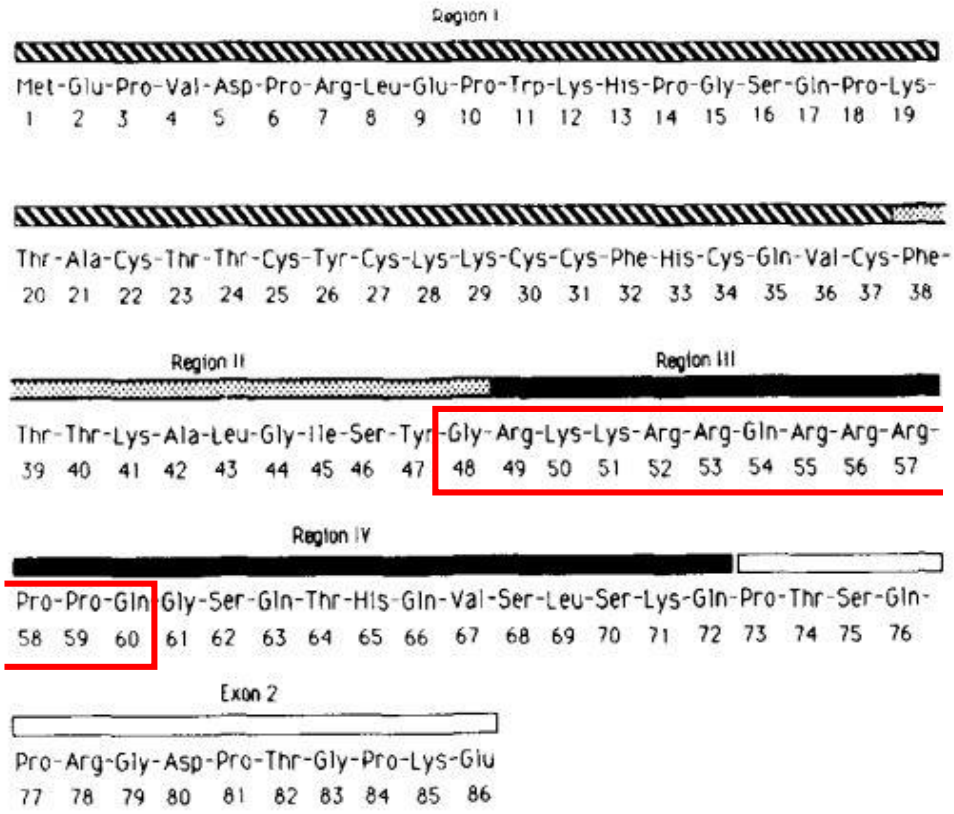


Figure 2. Amino Acid Sequence of HIV-1 Tat Protein (BRU Isolate)

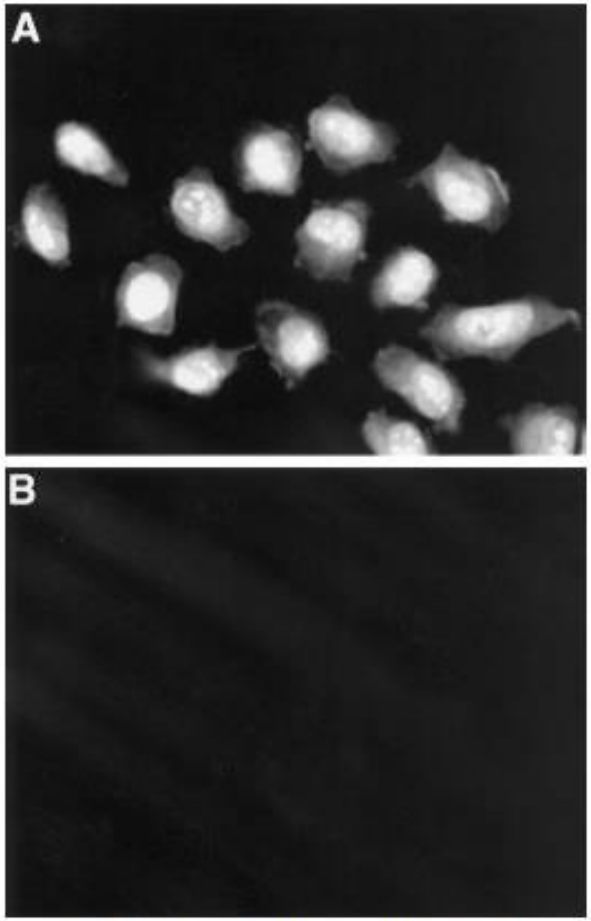
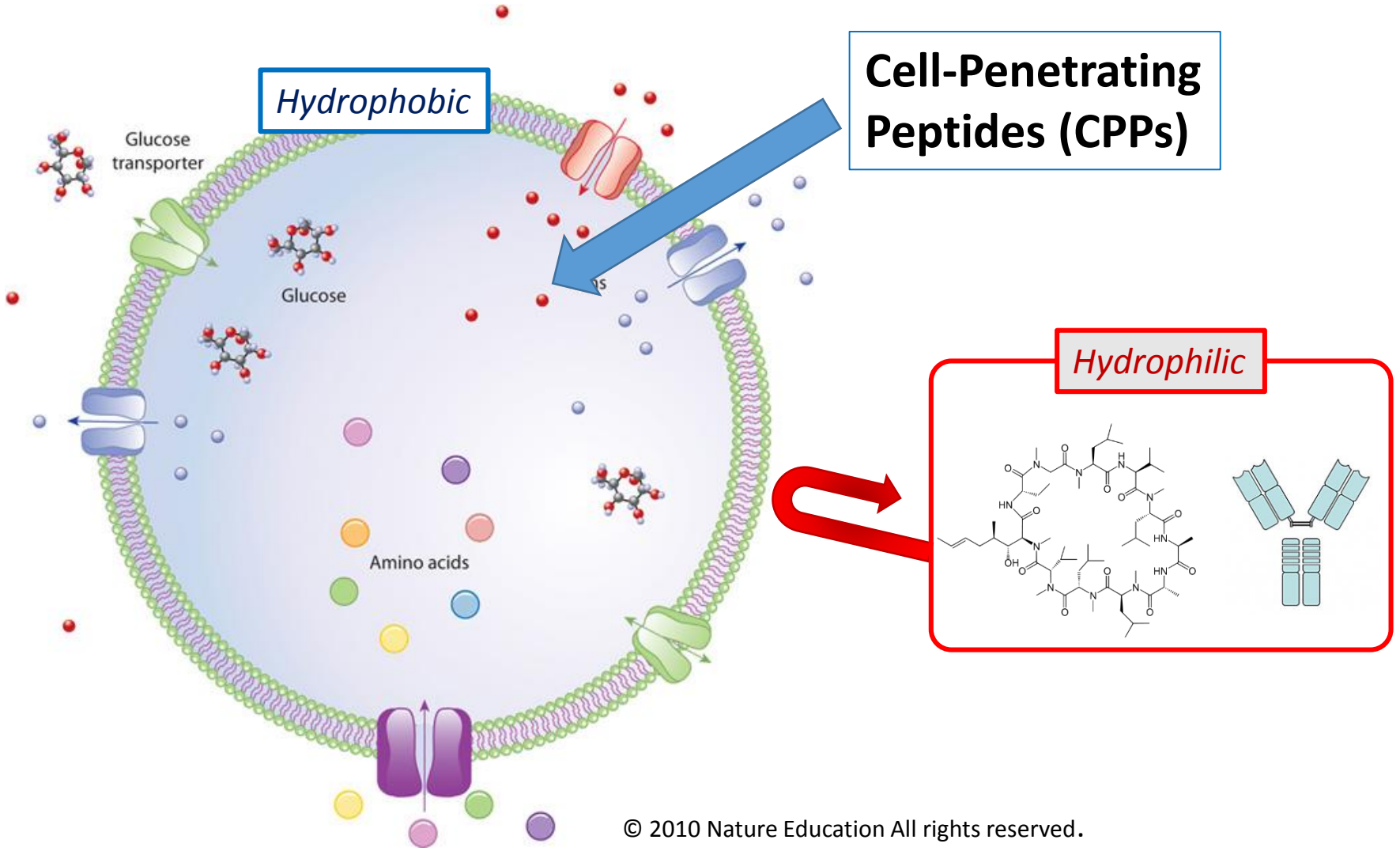


FIG. 4. **Effect of trypsinization on peptide uptake.** 3×10^5 HeLa cells were incubated with 5 μM fluorescein-labeled Tat-(48–60) peptide for 15 min at 37 °C (*panel A*) or with the same amount of peptide digested with trypsin for 1 h at 37 °C before incubation with cells (*panel B*).

Ref) Green, M. and Loewenstein, P.M. *Cell* 1988, 55, 1179–1188

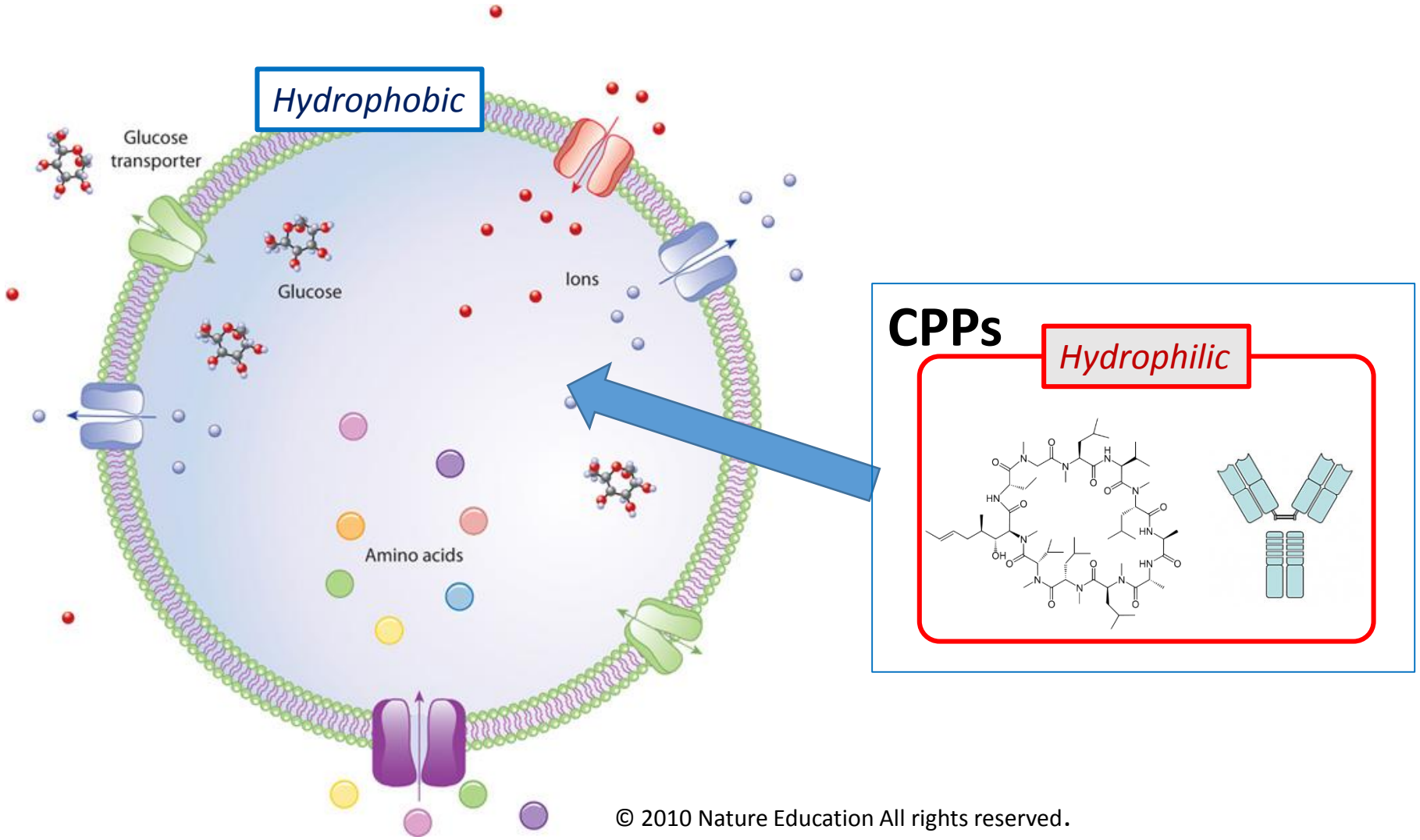
Ref) Vivès, E. et al. *J. Biol. Chem.* 1997, 272, 16010–16017

Strategy by CPPs



Specialized proteins in the cell membrane regulate the concentration of specific molecules inside the cell.

Strategy by CPPs



Specialized proteins in the cell membrane regulate the concentration of specific molecules inside the cell.

2. Classification of Cell-Penetrating Peptides (CPPs)

2-1. Cationic CPPs

2-2. Amphipathic CPPs

2-3. Hydrophobic CPPs

Various Cell-Penetrating Peptides (CPPs)

Table 1. Examples of CPPs and Their Sequences, Origins, and Physical–Chemical Properties

CPP name	Sequence	Origin	Class
HIV-1 TAT protein, TAT_{48-60}	GRKKRRQRRRPPQ	HIV-1 TAT protein	Cationic
HIV-1 TAT protein, TAT_{49-57}	RKKRRQRRR	HIV-1 TAT protein	Cationic
Penetratin, pAntp(43–58)	RQIKIWFQNRRMKWKK	Antennapedia *Drosophila melanogaster*	Cationic
Polyarginines	Rn	Chemically synthesized	Cationic
DPV1047	VKRGLKLRHVRPRVTRMDV	Chemically synthesized	Cationic
MPG	GALFLGFLGAAGSTMGAWSQPKKKRKV	HIV glycoprotein 41/ SV40 T antigen NLS	Amphipathic
Pep-1	KETWWETWWTEWSQPKKKRKV	Tryptophan-rich cluster/SV40 T antigen NLS	Amphipathic
pVEC	LLILRRRIRKQAHAHSK	Vascular endothelial cadherin	Amphipathic
ARF(1–22)	MVRRFLVTLRIRRACGPPRVRV	p14ARF protein	Amphipathic
BPrPr(1–28)	MVKSKIGSWILVLFVAMWSDVGLCKKRP	N terminus of unprocessed bovine prion protein	Amphipathic
MAP	KLALKLALKALKAALKLA	Chemically synthesized	Amphipathic
Transportan	GWTLNSAGYLLGKINLKALAALAKKIL	Chimeric galanin–mastoparan	Amphipathic
p28	LSTAADMQGVVTDGMASGLDKDYLKPDD	Azurin	Amphipathic
VT5	DPKGDPKGVTVTVTVTVTGKGDPKPD	Chemically synthesized	Amphipathic
Bac 7 (Bac_{1-24})	RRIRPRPPRLPRPRPRPLPFPRPG	Bactenecin family of antimicrobial peptides	Amphipathic
C105Y	CSIPPEVKFNKPFVYLI	α1-Antitrypsin	Hydrophobic
PFVYLI	PFVYLI	Derived from synthetic C105Y	Hydrophobic
Pep-7	SDLWEMMMVSLACQY	CHL8 peptide phage clone	Hydrophobic

① Cationic

② Amphipathic

③ Hydrophobic

Ref) Daniela Rossi et al. *Trends in Pharmacological Sciences* 2017, 38, 406-424

2. Classification of Cell-Penetrating Peptides (CPPs)

2-1. Cationic CPPs

2-2. Amphipathic CPPs

2-3. Hydrophobic CPPs

① Cationic CPPs

TAT (Green, M. and Loewenstein in 1988)

Polyarginines *(Futaki, S. in 2001)*

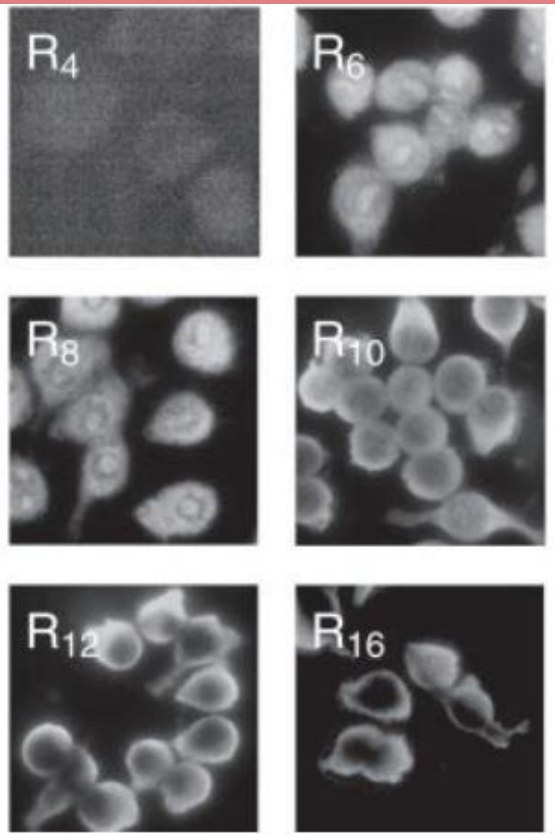


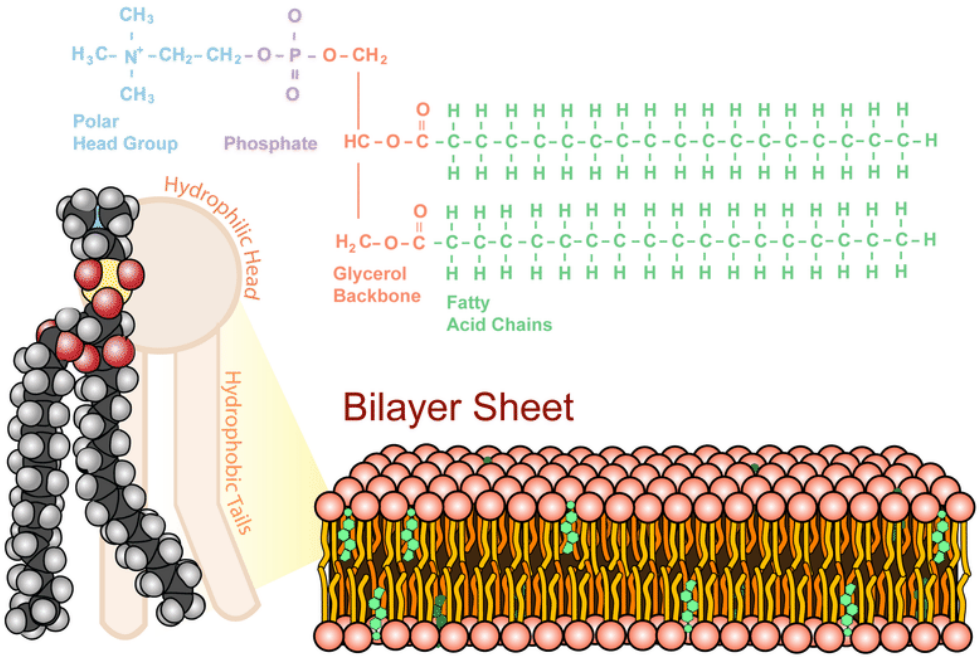
FIG. 1. **Structure of arginine-rich peptides used in this study.** C-terminal cysteine amide (*C**) was fluorescein-labeled for monitoring the internalization of the peptides by fluorescence microscopy.

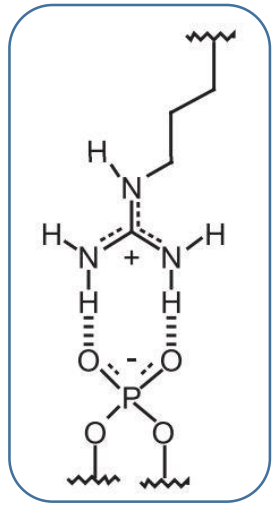
Ref) Futaki, S. et al. *J. Biol. Chem.* 2001, 276, 5836–5840

① Cationic CPPs

Abundant basic amino acids will be <u>cationic</u>
under physiological environment.
So they interact with phospholipid head groups of
cell membrane.

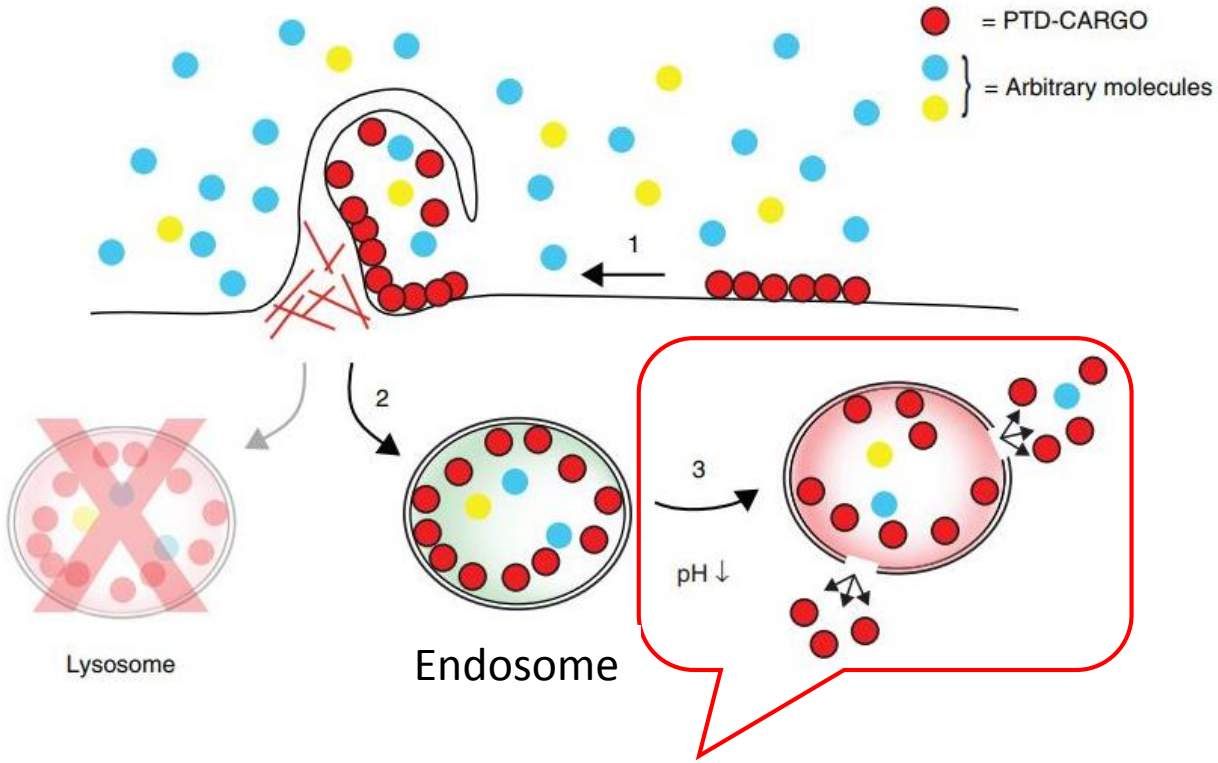
Especially, arginine can form hydrogen bond with it.





Ref) Morris, M.C. et al. *Biol. Cell* 2008, 100, 201–217

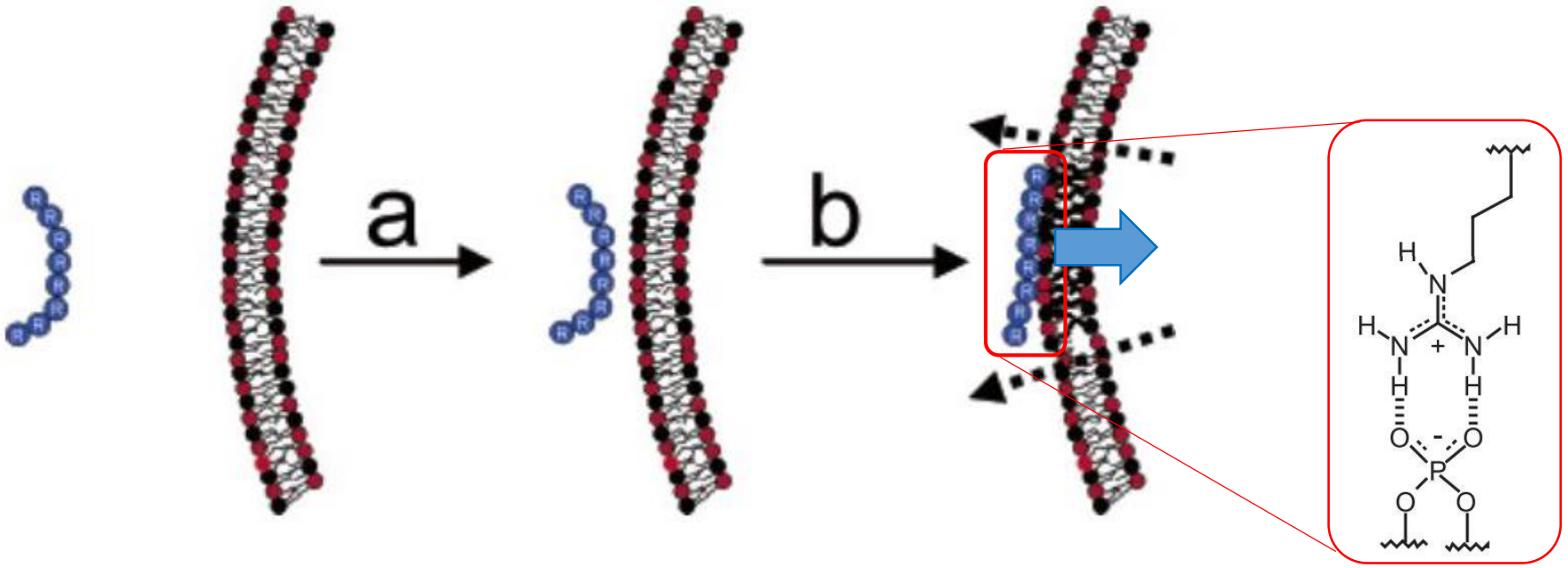
Endocytosis



CPPs can be more positive and interact with the negatively charge components of the endosomal membrane.
This binding causes stiffening of the membrane, determining its rupture and the release of the vesicle's contents.

Ref) van den Berg, A. and Dowdy, S.F. *Curr. Opin. Biotechnol*. 2011, 22, 888–893

Polyarginines

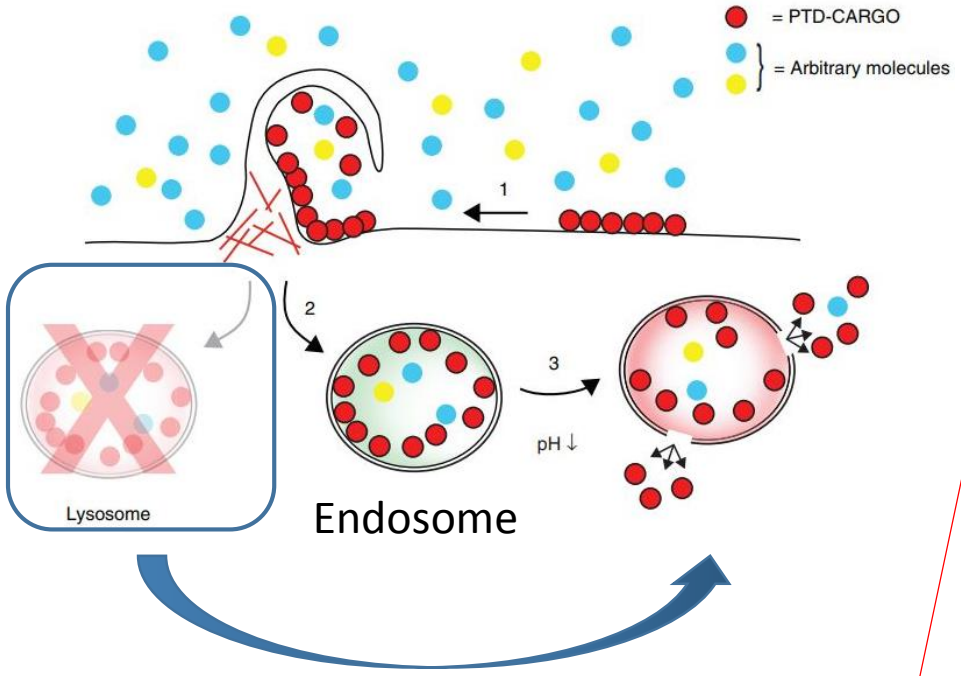


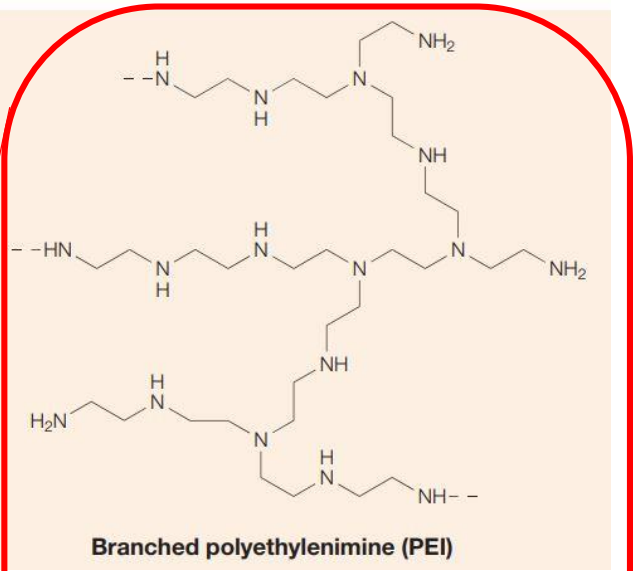
Electrostatic interaction : 25 - 30%
Hydrogen bond : **70 – 75%**

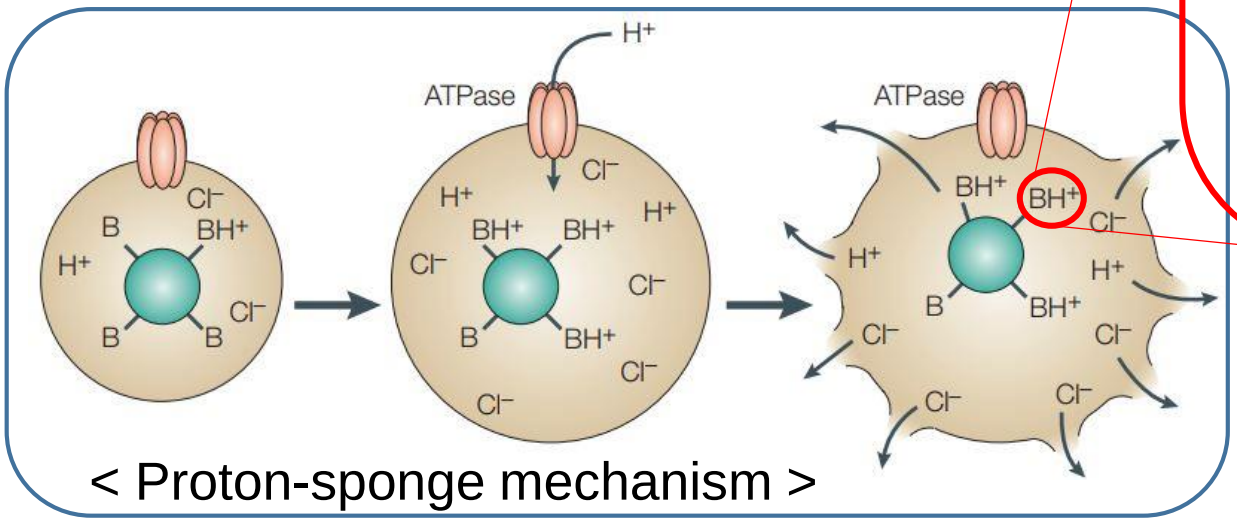
Polyarginines can interact with the membrane by hydrogen bond. So, arginine-rich CPPs may enter the cell via a nonendocytotic mechanism.

Ref) Hitz, T. et al. *Biochemistry* 2006, 45, 5817–5829

Escape from endosome : Proton-sponge mechanism



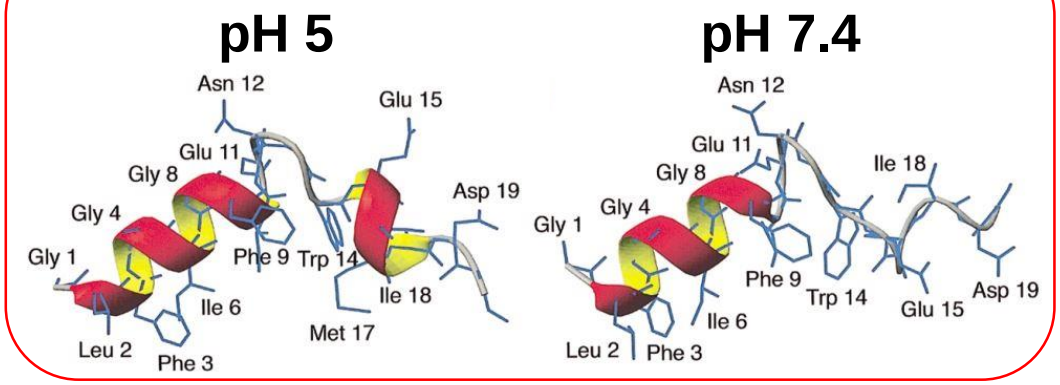


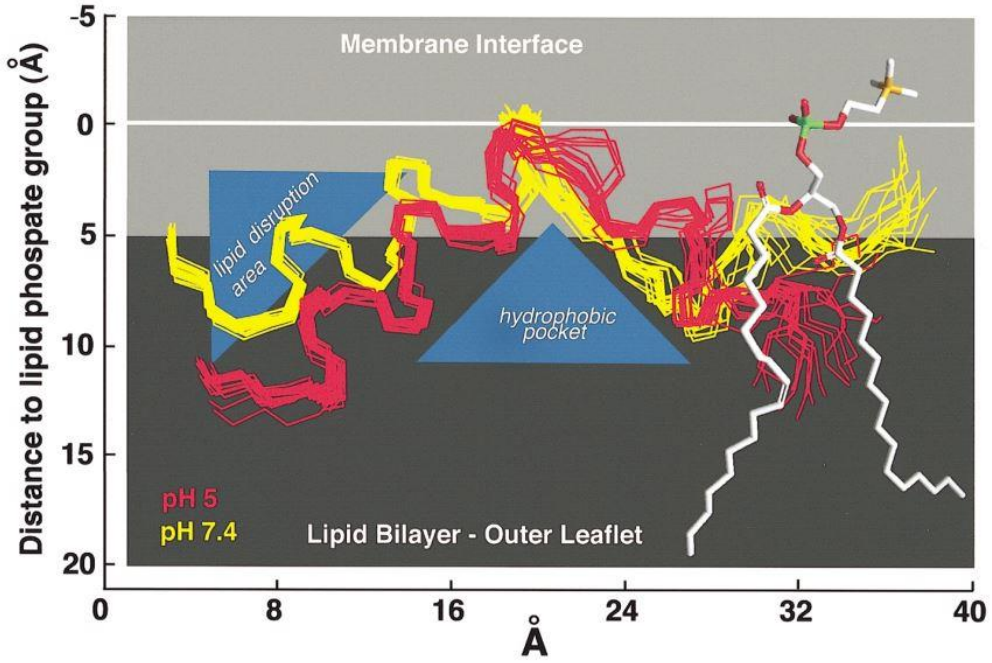


Ref) D. W. Pack, A. S. Hoffman, S. Pun, and P. S. Stayton. *Nat. Rev. Drug Discov.* 2005, 4(7), 581–593

Escape from endosome : TAT-HA2

HA2 peptide





N-terminal 20 amino acids of the influenza virus hemagglutinin protein

TAT HA2

RRRQRRKKRG GDIMGEWGNEIFGAIAGFLG

The deeper insertion of the V-shaped structure destabilizes the membrane. So, TAT-HA2-fusion protein can escape from endosome.

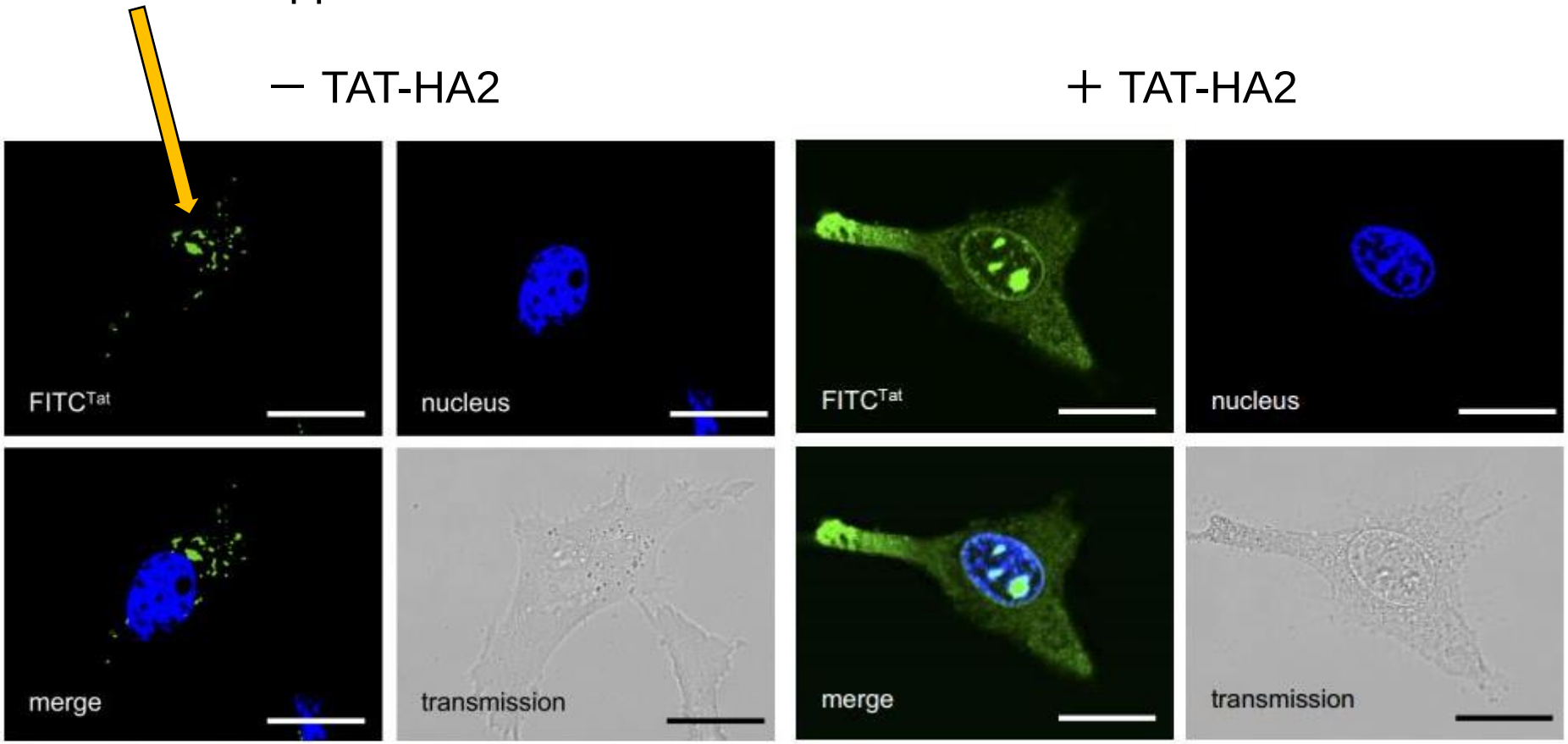
Ref) Han, X., Bushweller, J.H., Cafiso, D.S., Tamm, L.K. *Nat. Struct. Biol*. 2001, 8, 715–720

Ref) David, Y., Vila-Perello, M., Verma, S., Muir, T. W. *Nat. Chem.* 2015, 7, 394

Escape from endosome ： agents to help endosomal escape

Only using TAT-HA2 as a reagent can help endosomal escape.

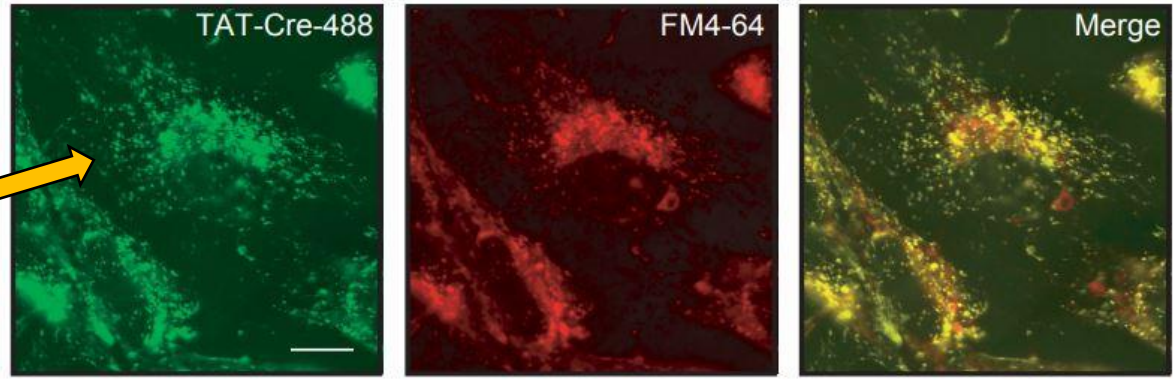
FITC-TAT trapped in endosome.

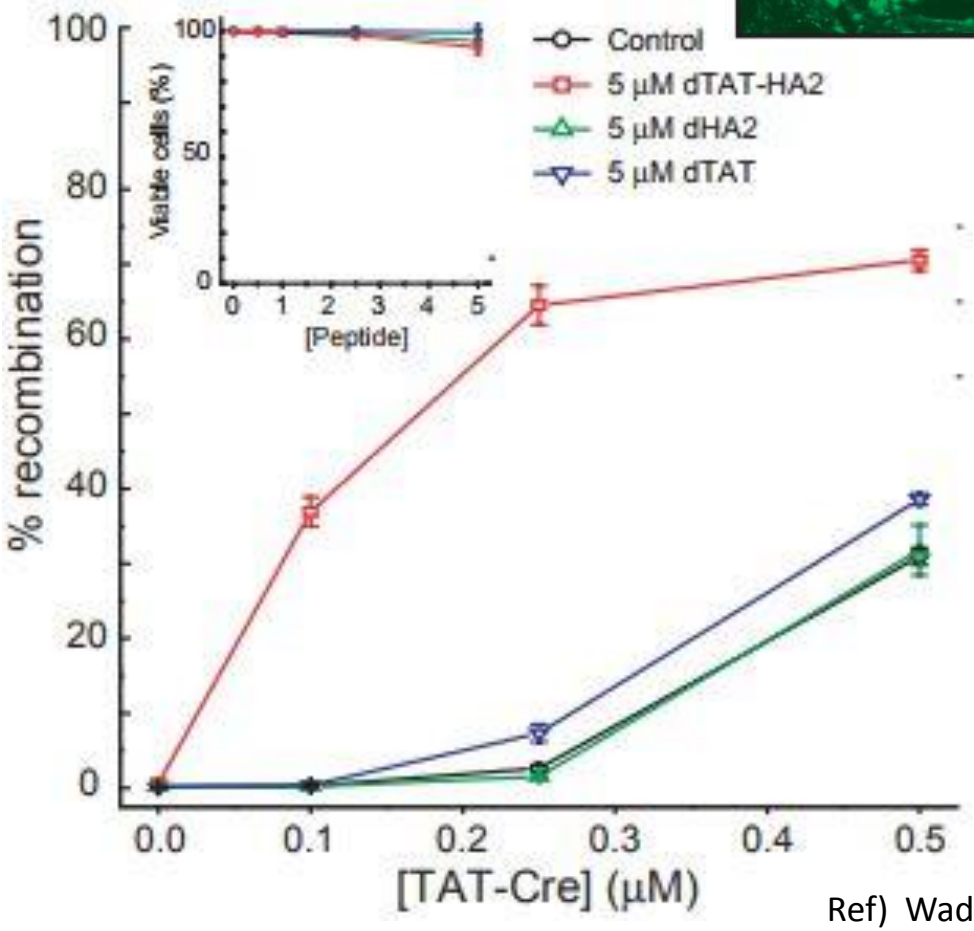


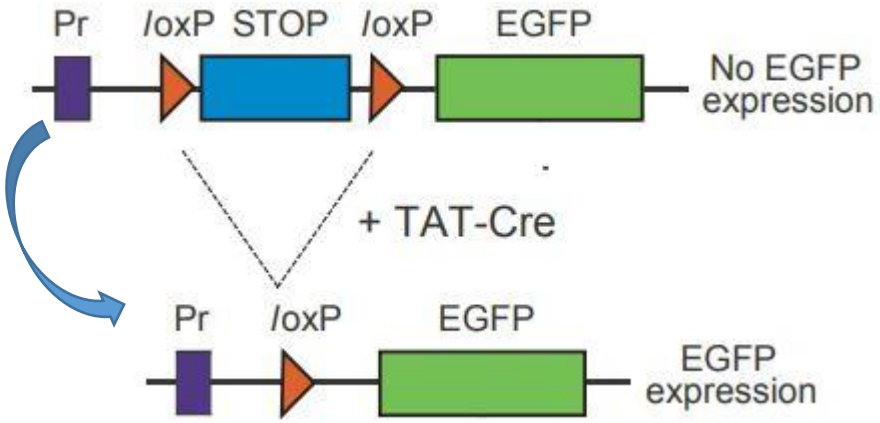
Ref) T. Sugita et al. *Biochemical and Biophysical Research Communications* 2007, 363, 1027–1032

Escape from endosome : agents to help endosomal escape

TAT-Cre trapped in endosome.



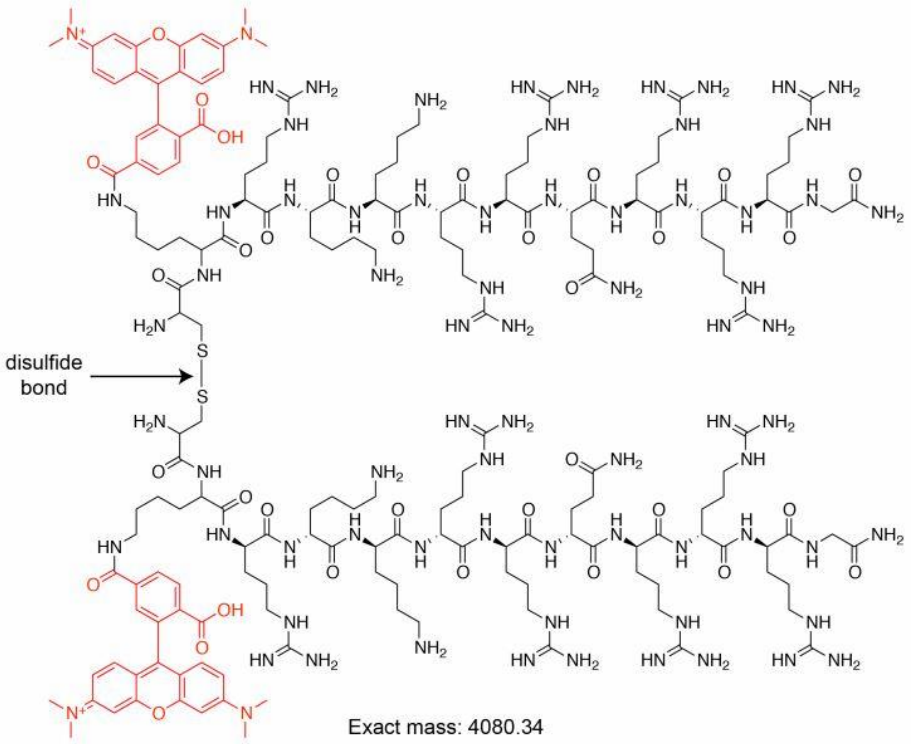


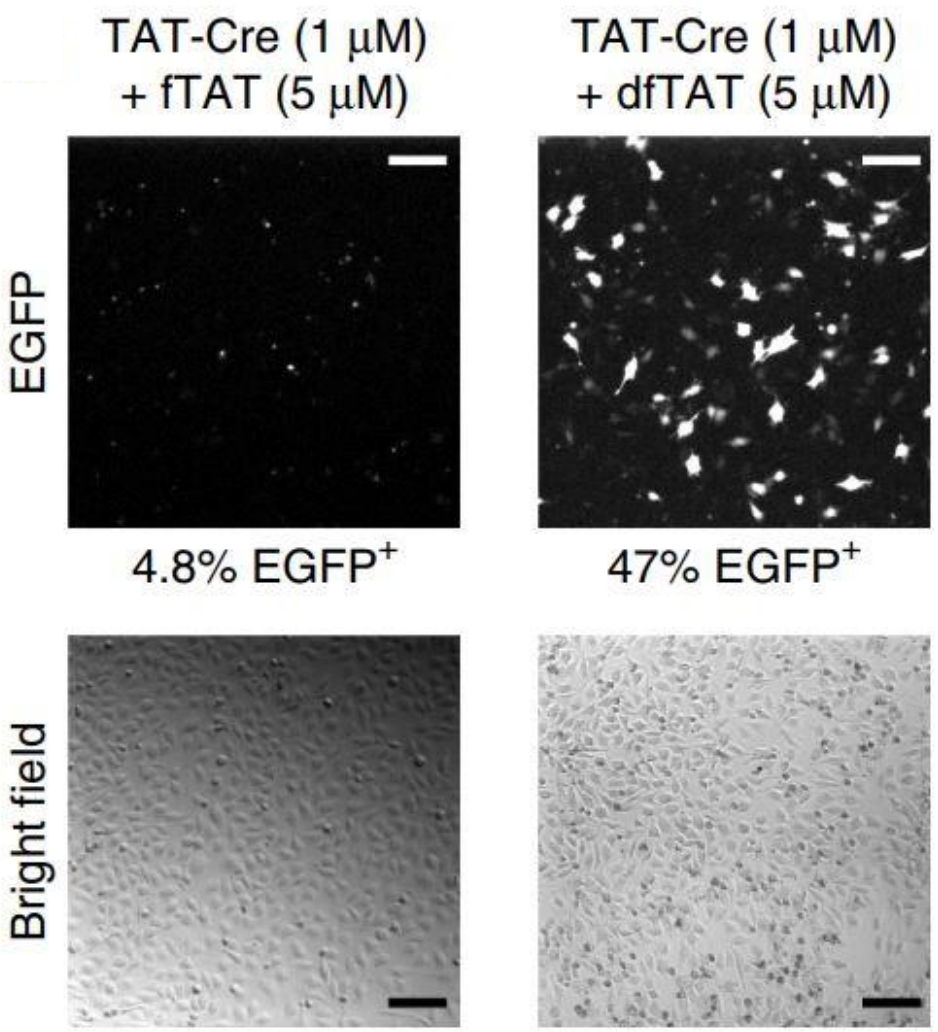


When TAT-Cre enters in cells, it recombine EGFP reporter gene.

Ref) Wadia, J. S., Stan, R. V., Dowdy, S. F. *Nat. Med*. 2004, 10, 310-315

Escape from endosome : agents to help endosomal escape



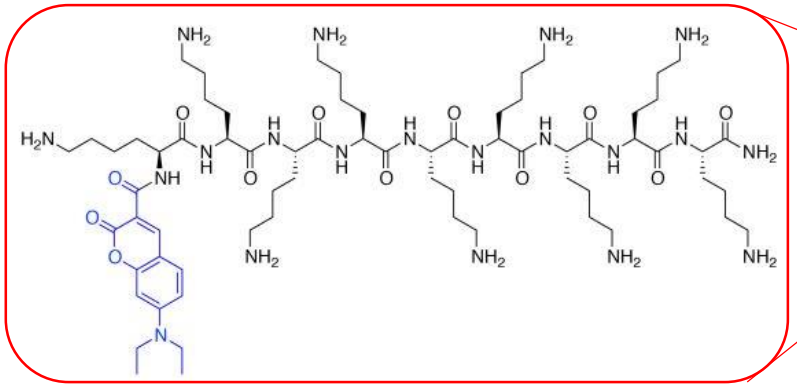


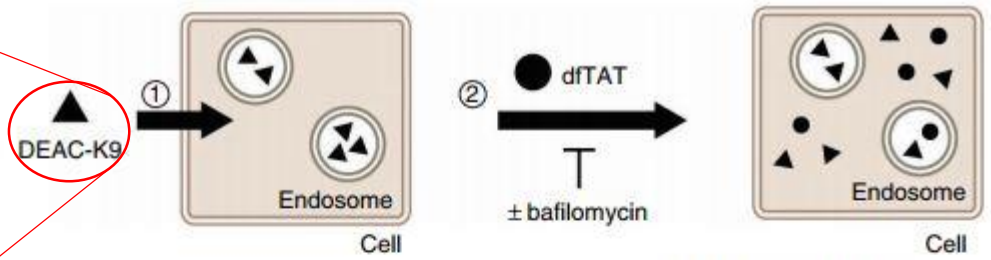
dfTAT = *dimeric* fluorescent TAT

dfTAT is more endosomolytic and cause lysis of endosomal membrane than monomeric TAT.

Ref) J. P. Pellois et al. *Nat. Meth*. 2014, 11, 861-867

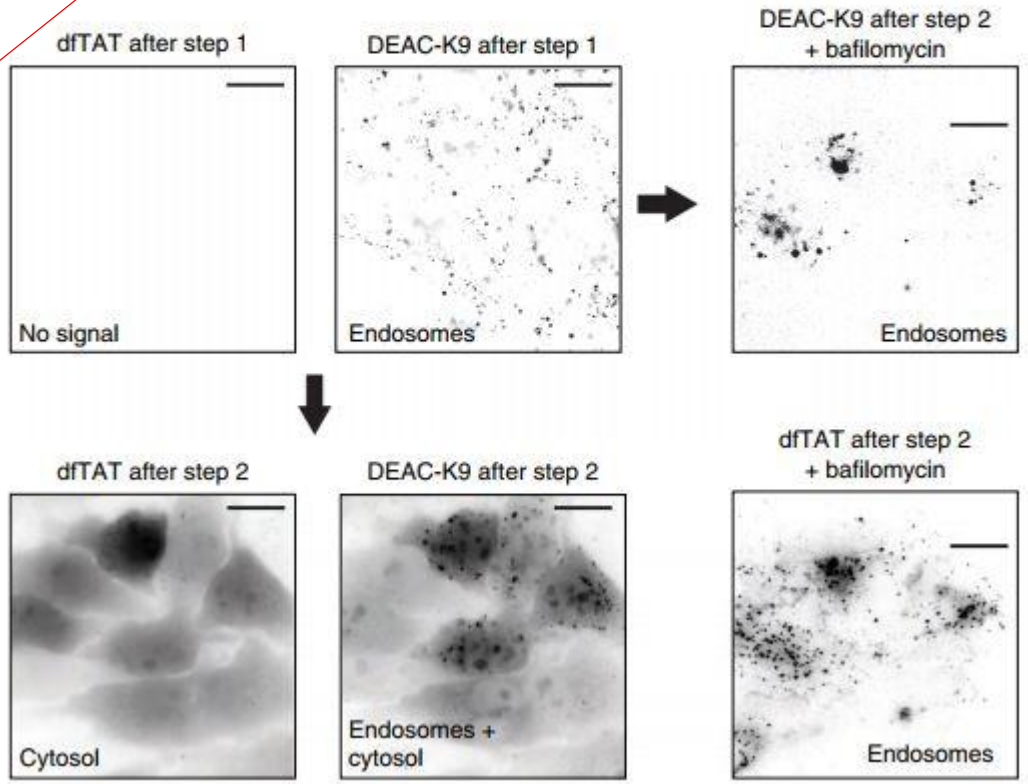
Escape from endosome ： agents to help endosomal escape





Bafilomycin (V-ATPase inhibitor) inhibited the delivery by dfTAT.

Endocytosis and endosomal acidification are important for cytosolic penetration.



Ref) J. P. Pellois et al. *Nat. Meth*. 2014, 11, 8, 861-867

2. Classification of Cell-Penetrating Peptides (CPPs)

2-1. Cationic CPPs

2-2. Amphipathic CPPs

2-3. Hydrophobic CPPs

② Amphipathic CPPs

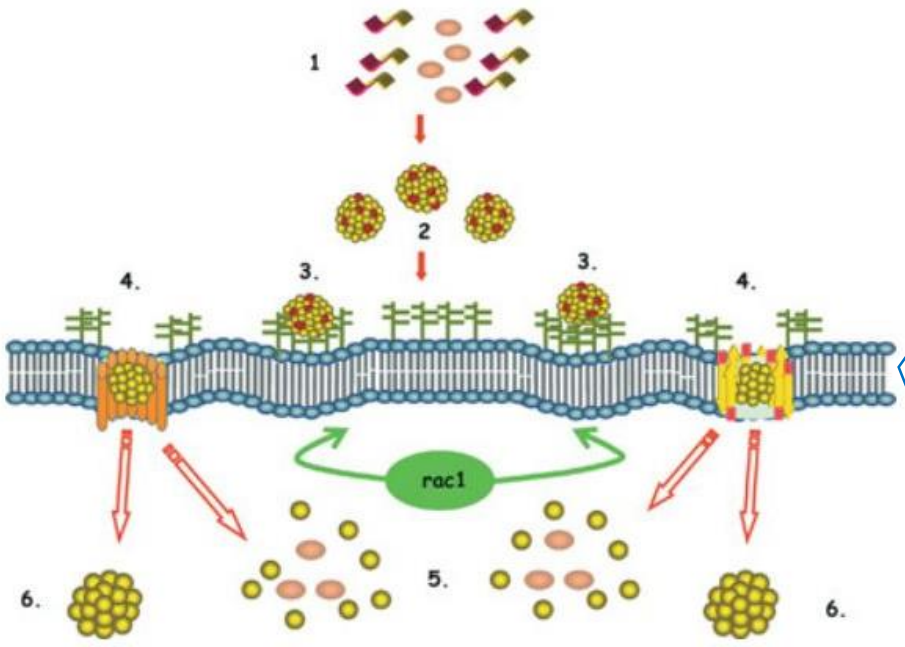
Amphipathic CPPs contain both polar (hydrophilic) and nonpolar (hydrophobic) regions of amino acids.

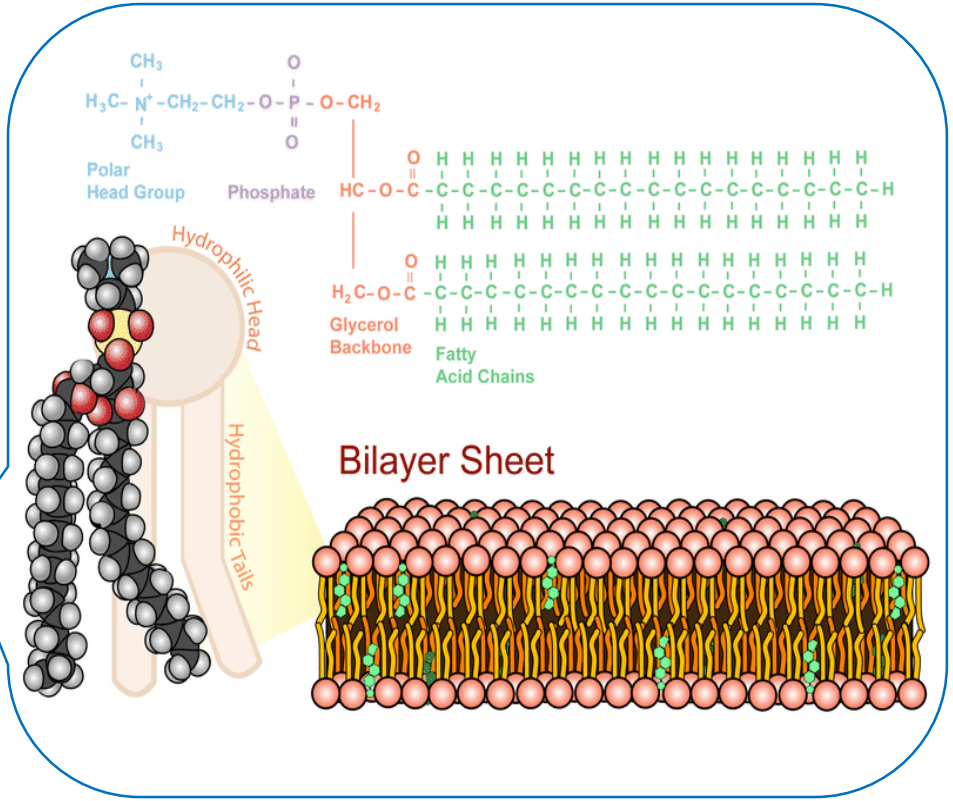
CPP name	Sequence	Origin
MPG	GALFLGFLGAAGSTMGAWSQPKKKRKV	HIV glycoprotein 41/ SV40 T antigen NLS
Pep-1	KETWWETWWTEWSQPKKKRKV	Tryptophan-rich cluster/SV40 T antigen NLS
pVEC	LLILRRRIRKQAHAHSK	Vascular endothelial cadherin
ARF(1–22)	MVRRFLVTLRIRRACGPPRVRV	p14ARF protein
BPrPr(1–28)	MVKSKIGSWILVLFVAMWSDVGLCKKRP	N terminus of unprocessed bovine prion protein
MAP	KLALKLALKALKAALKLA	Chemically synthesized
Transportan	GWTLNSAGYLLGKINLKALAALAKKIL	Chimeric galanin–mastoparan
p28	LSTAADMQGVVTDGMASGLDKDYLKPDD	Azurin
VT5	DPKGDPKGVTVTVTVTVTGKGDPKPD	Chemically synthesized
Bac 7 (Bac $_{1-24}$)	RRIRPRPPRLPRPRPRPLPFPRPG	Bactenecin family of antimicrobial peptides

Ref) Daniela Rossi et al. *Trends in Pharmacological Sciences* 2017, 38, 406-424

② Amphipathic CPPs

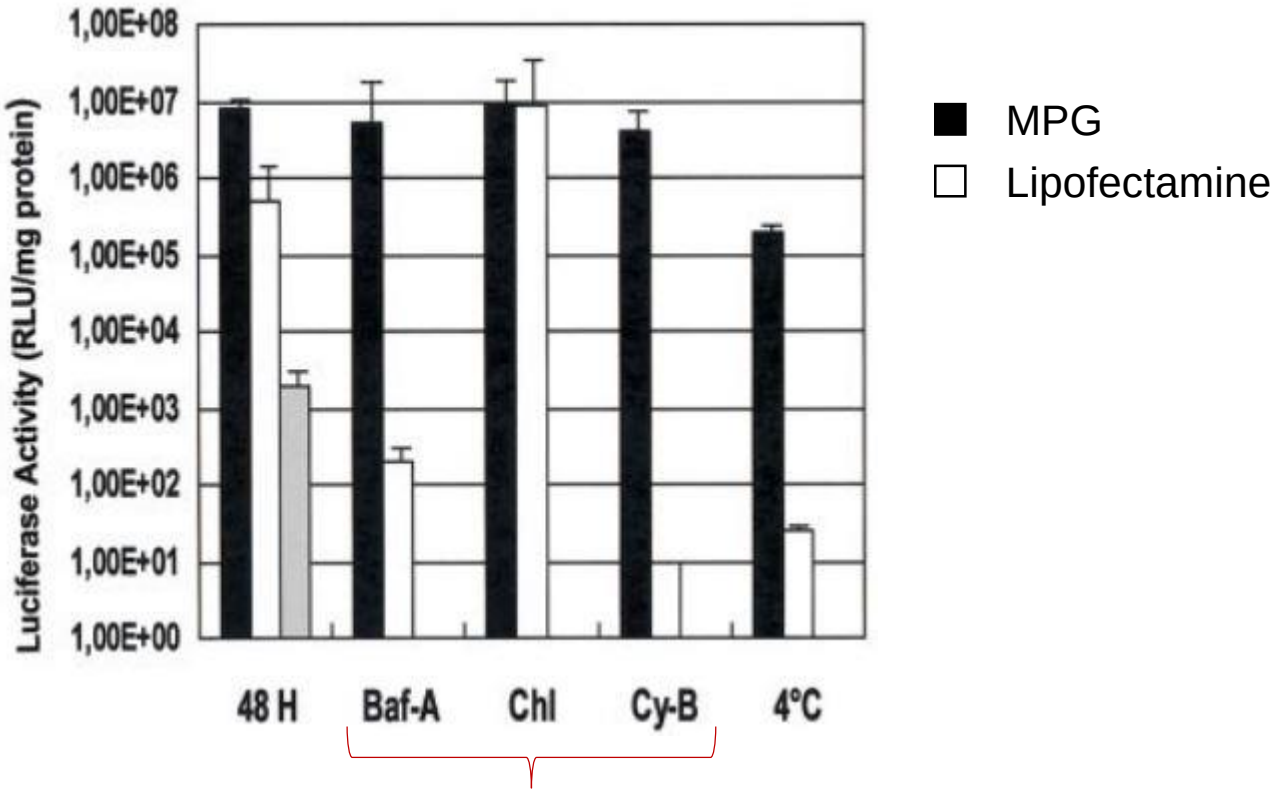
Amphipathic CPPs can enter the cell directly
in addition to endocytosis.





Ref) Morris, M.C. et al. *Biol. Cell* 2008, 100, 201–217

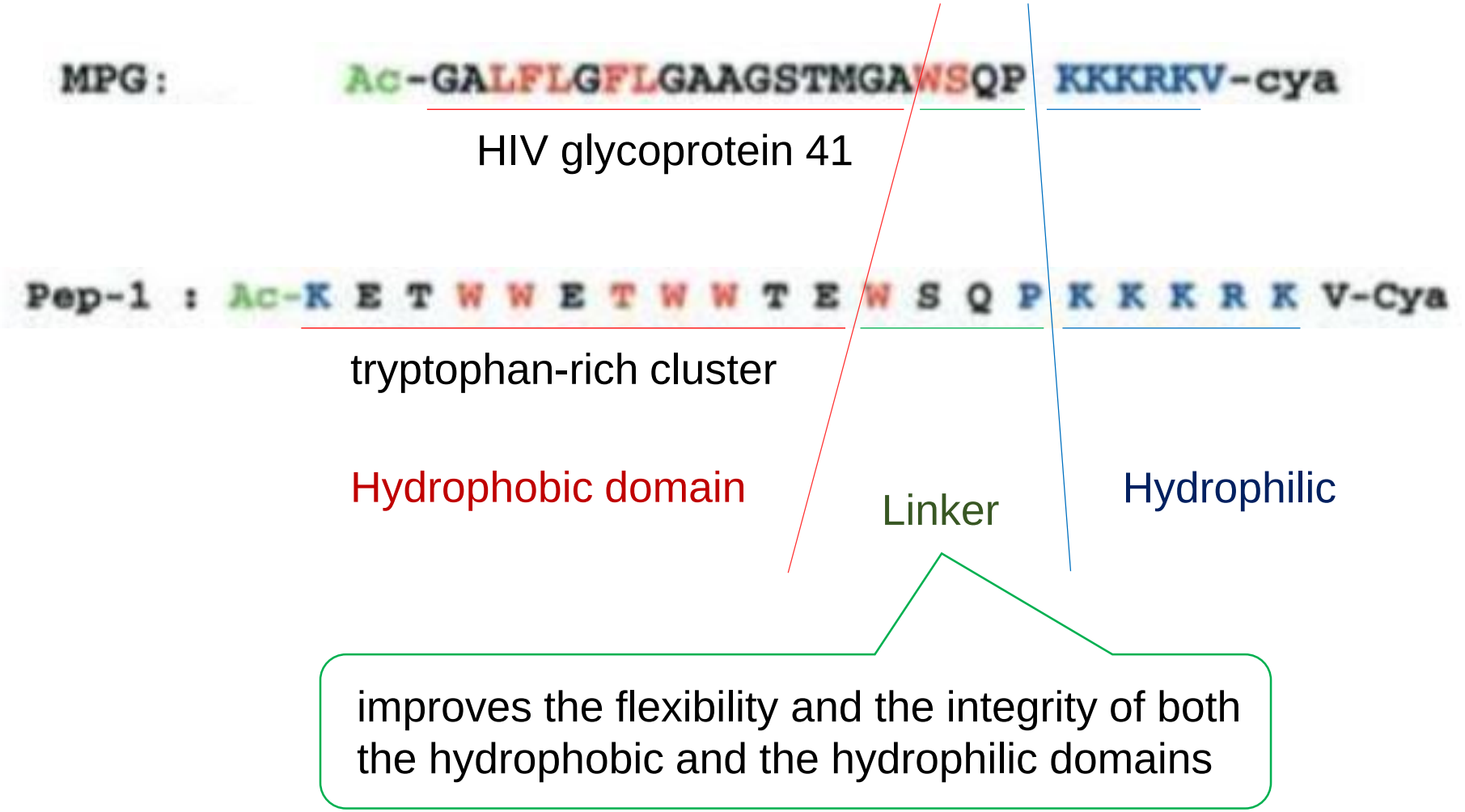
Amphipathic CPPs can enter cells directly



Inhibitors that interfere with the endosomal pathway

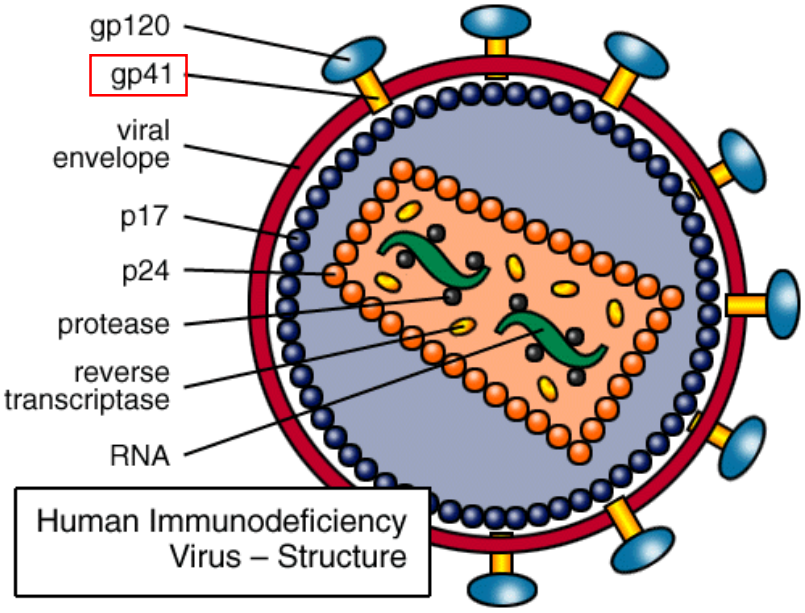
Ref) Gilles Divita et al. *Nuc. Acid. Res.* 2003, 31, 11, 2717-2724

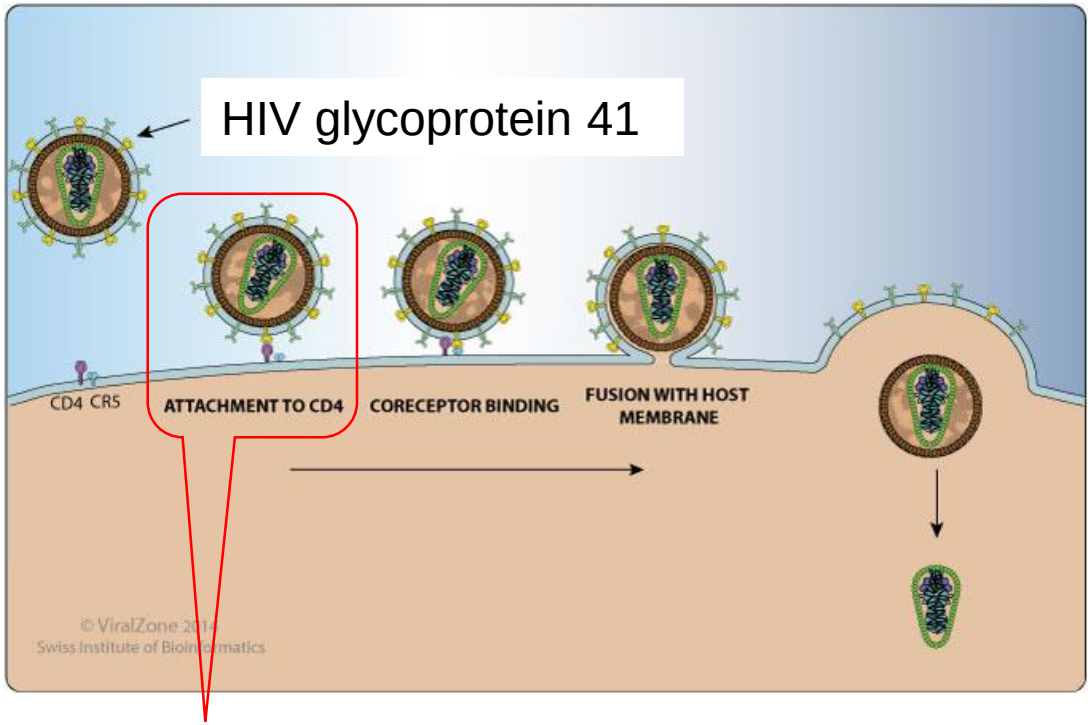
MPG and Pep-1



Ref) Morris, M.C. et al. *Biol. Cell* 2008, 100, 201–217
Ref) Gilles Divita et al. *Nuc. Acid. Res.* 2003, 31, 11, 2717-2724

HIV glycoprotein 41

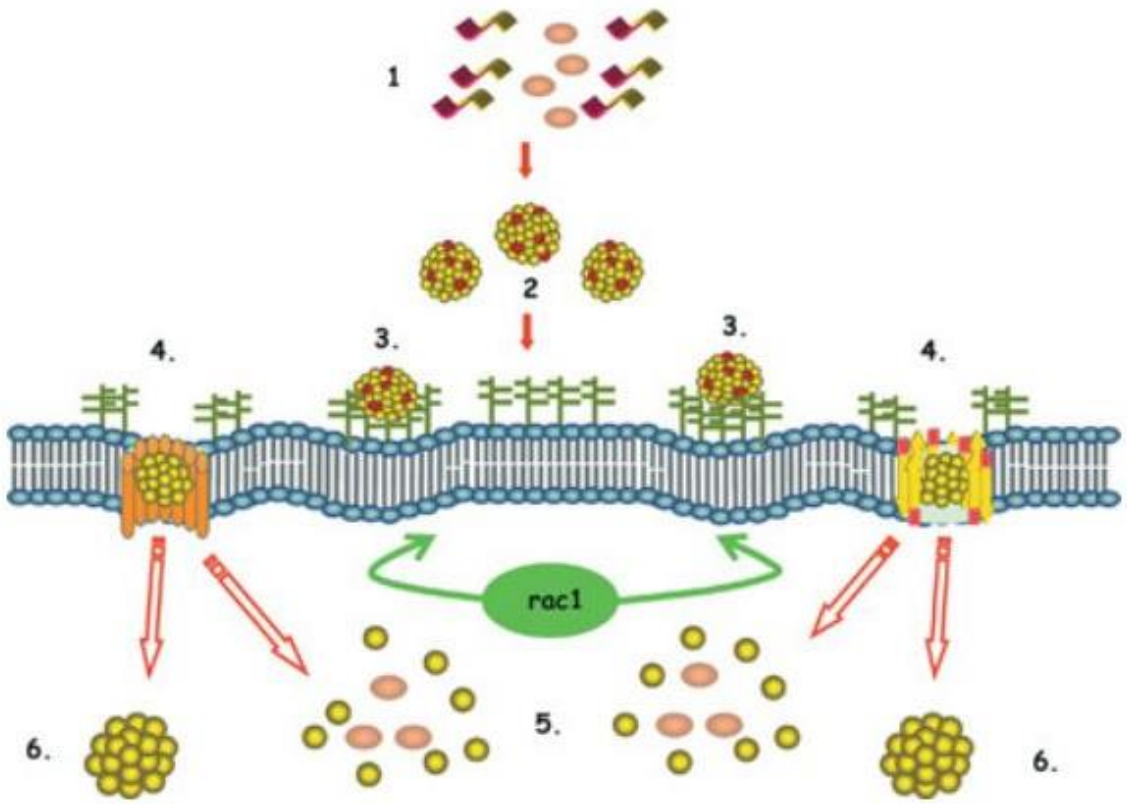




HIV gp41 interacts with the host cellular membrane.

Ref) Morris, M.C. et al. *Biol. Cell* 2008, 100, 201–217
Ref) Gilles Divita et al. *Nuc. Acid. Res.* 2003, 31, 11, 2717-2724

Amphipathic CPPs can enter the cell directly



(1) Formation of the carrier—cargo complexes.
(2) Interaction of the carrier–cargo nanoparticles with the cell surface proteoglycans
(3) Interaction with the glycans and phospholipid head groups
(4) Direct interaction with the lipid phase of the cell membrane
(5) Complexes are released into the cytoplasm
or (6) is targeted to the nucleus or to specific organelles.

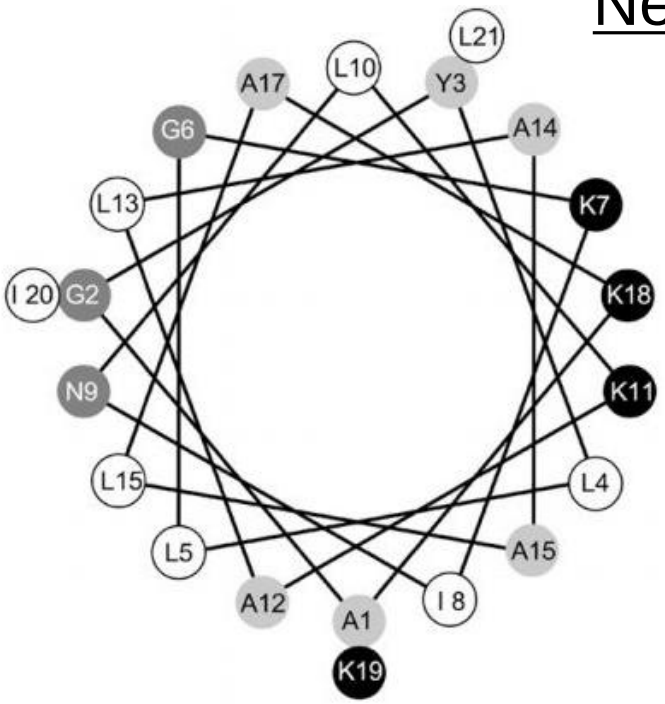
Ref) Morris, M.C. et al. *Biol. Cell* 2008, 100, 201–217

TP10

TP10 : AGYLLG | K | INLKALAALAKKIL-amide

Galanin | Mastoparan

Neuropeptide

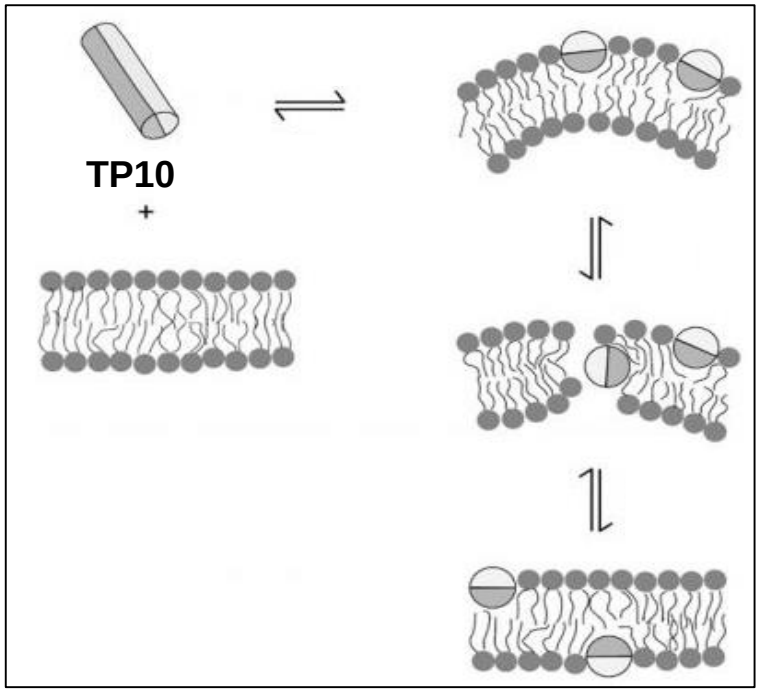


Sectional view of TP10

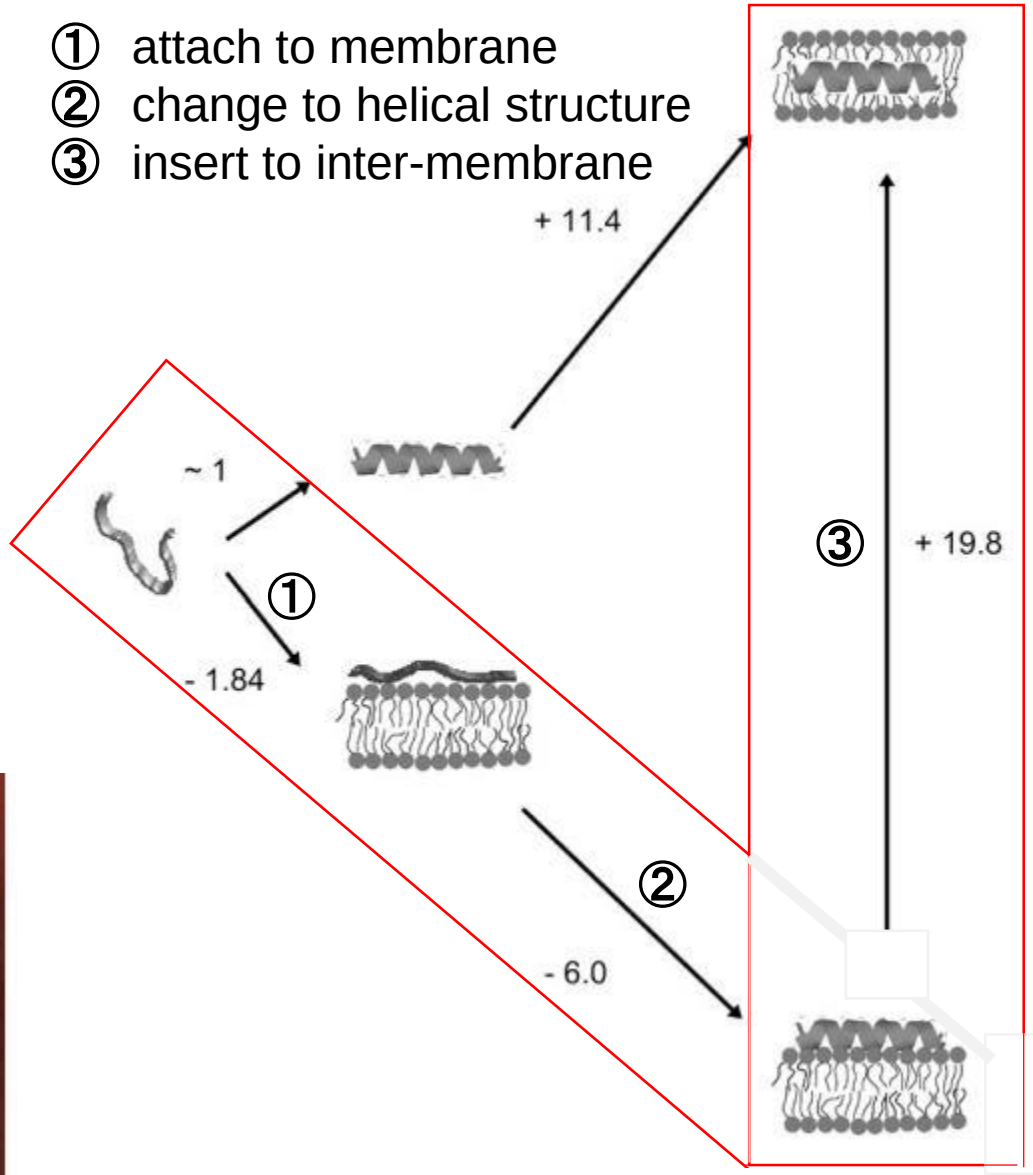
Helical structure

Ref) Ulo Langel et al. *The FASEB Journal* 2018, 12, 1, 67-77

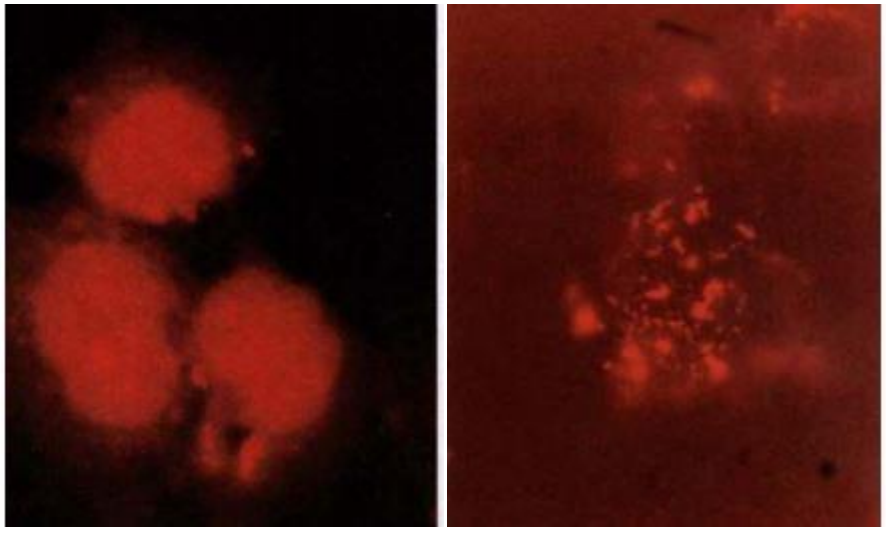
Mechanism of TP10 / membrane interaction



① attach to membrane
② change to helical structure
③ insert to inter-membrane



＋ TP10　　－ TP10



Ref) Ulo Langel et al. *Nat. Bio. Tech.* 1998, 16, 857-861

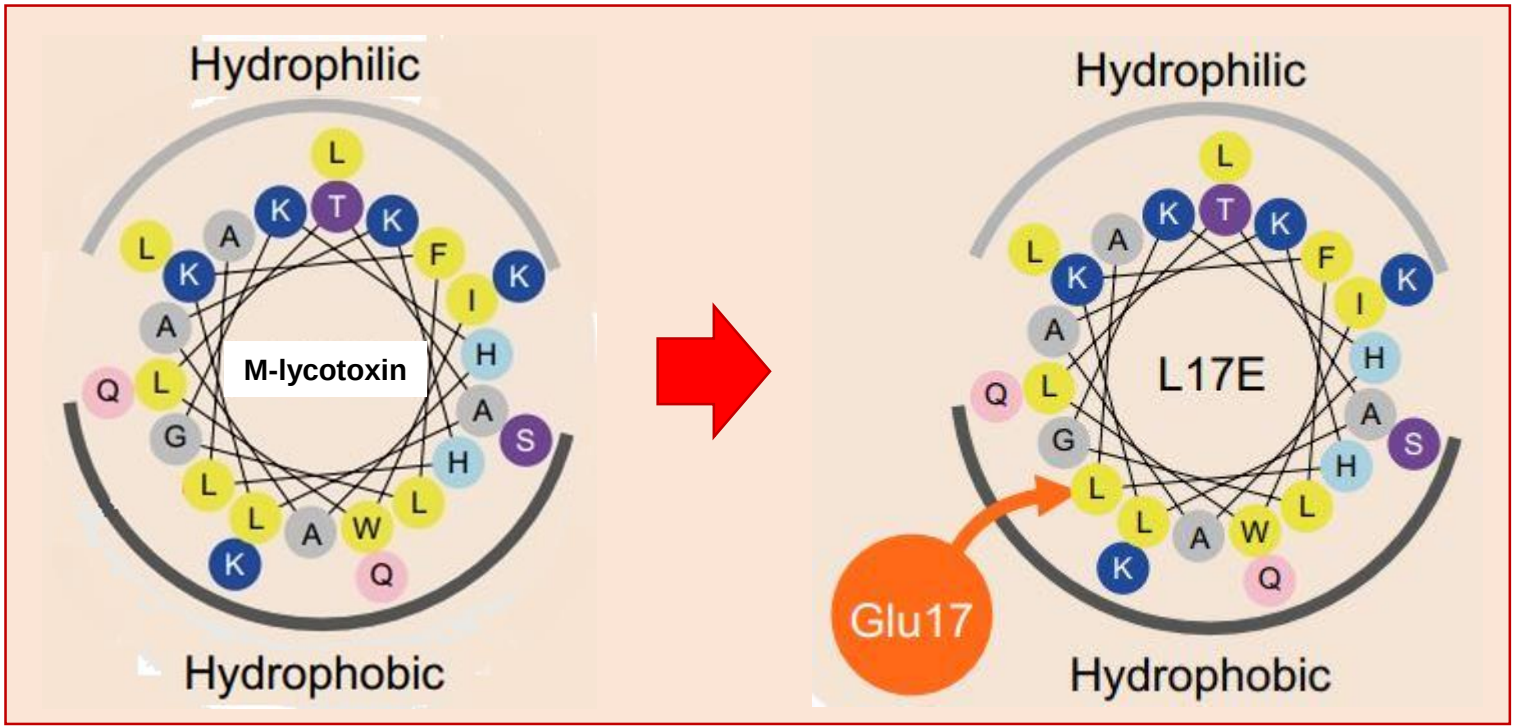
Ref) Ulo Langel et al. *Biophysical Journal* 2007, 92, 2434-2444

L17E : agents to help endosomal escape

L17E is an agent to help endosomal escape derived from Amphipathic CPP.

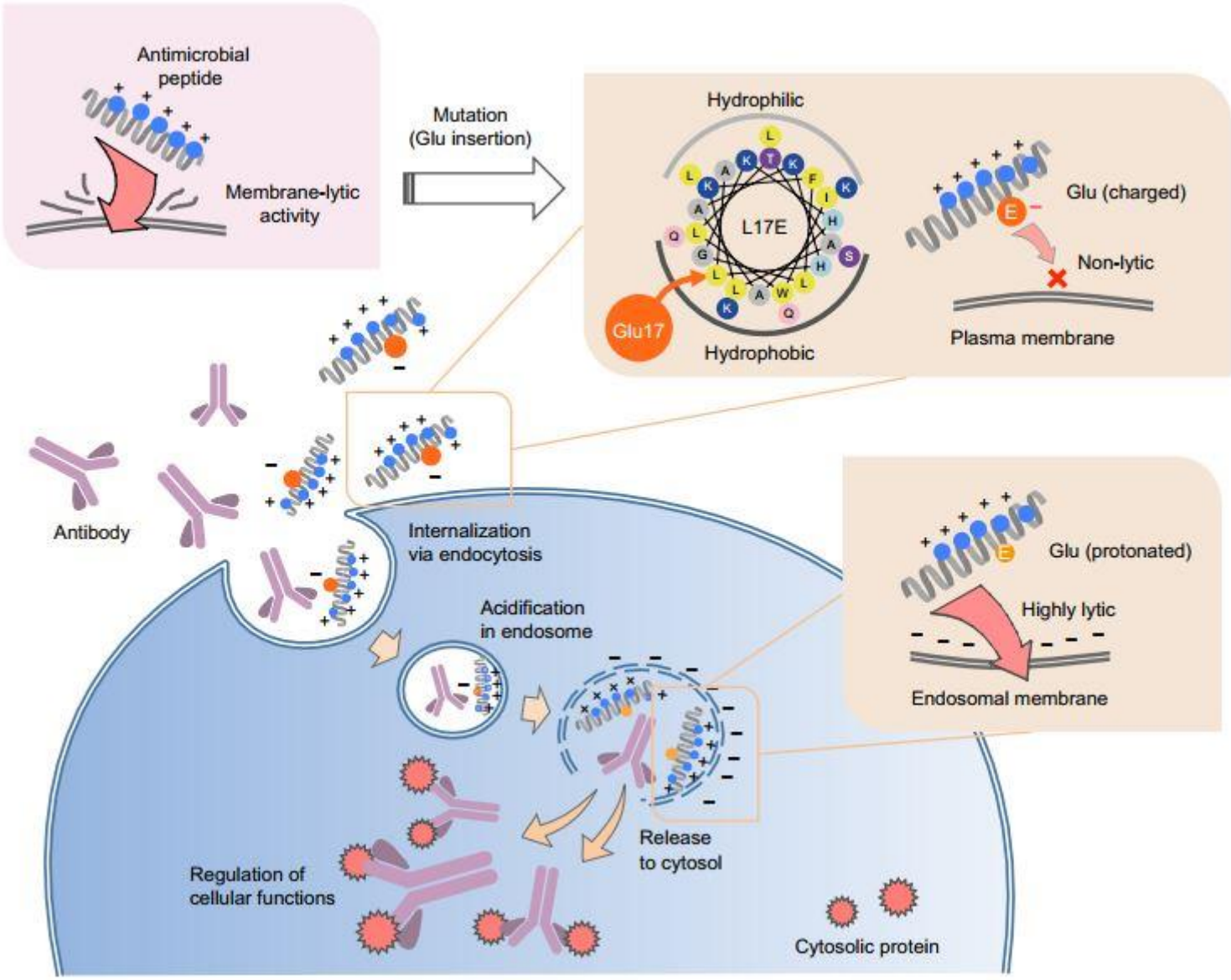
M-lycotoxin, which disrupts cell membrane, derived from the venom of the wolf spider Lycosa carolinensis27.

Prof. Futaki @ Kyoto Uni.



Ref) Shiroh Futaki et al. *Nat. Chem*. 2017, 9, 751–761

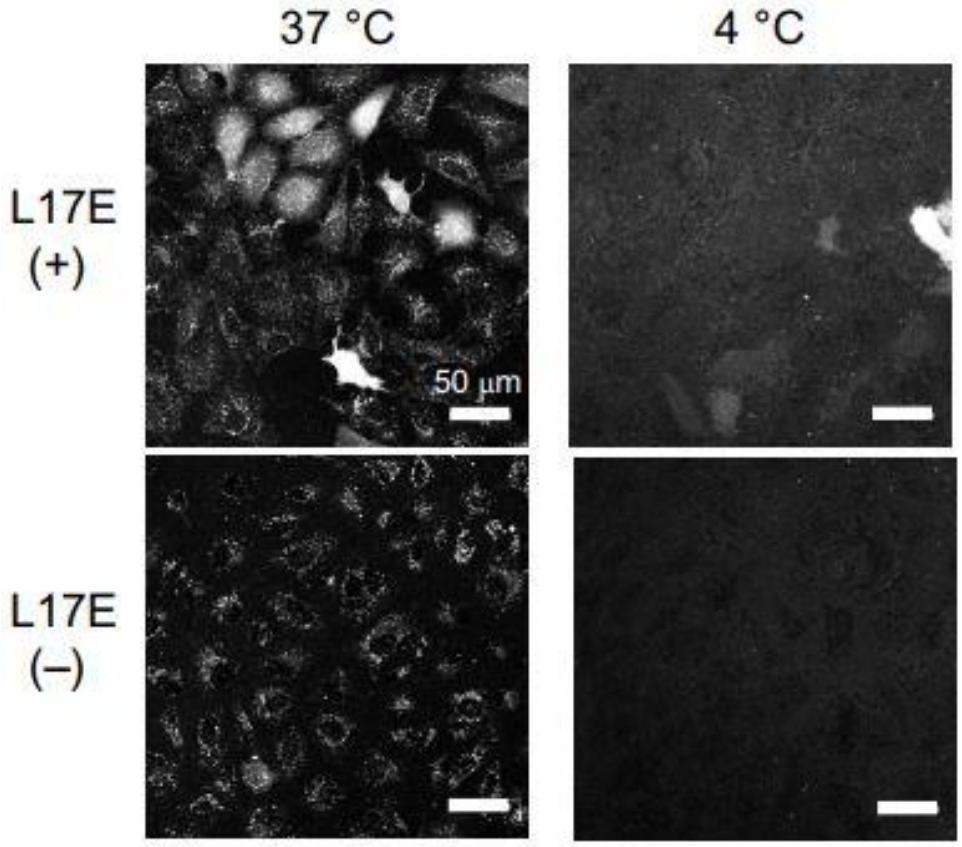
L17E : agents to help endosomal escape



Ref) Shiroh Futaki et al. *Nat. Chem*. 2017, 9, 751–761

L17E : agents to help endosomal escape

Alexa488–dextran



L17E mediated cytosolic delivery.

Ref) Shiroh Futaki et al. *Nat. Chem*. 2017, 9, 751–761

2. Classification of Cell-Penetrating Peptides (CPPs)

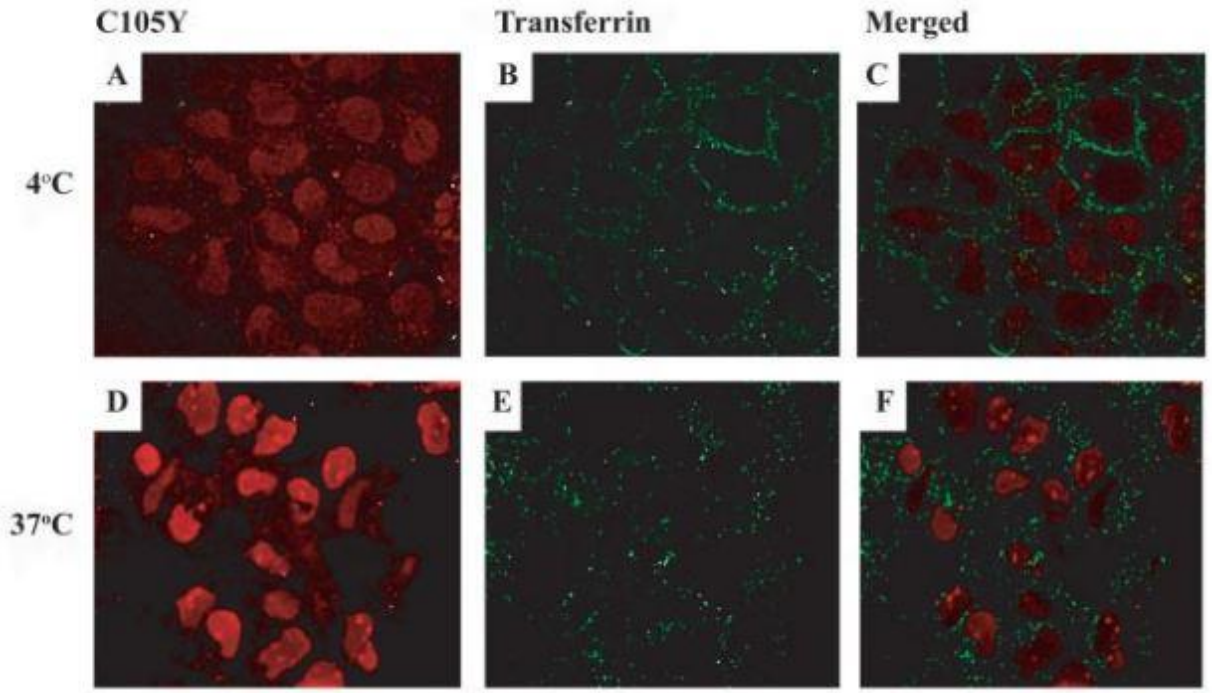
2-1. Cationic CPPs

2-2. Amphipathic CPPs

2-3. Hydrophobic CPPs

③ Hydrophobic CPPs

As yet only a limited number of hydrophobic peptides has been discovered and their internalization mechanisms have been poorly studied compared with the cationic and amphipathic classes.
However, it has been proposed that this family of peptides could spontaneously translocate across membranes in an energy-independent manner.

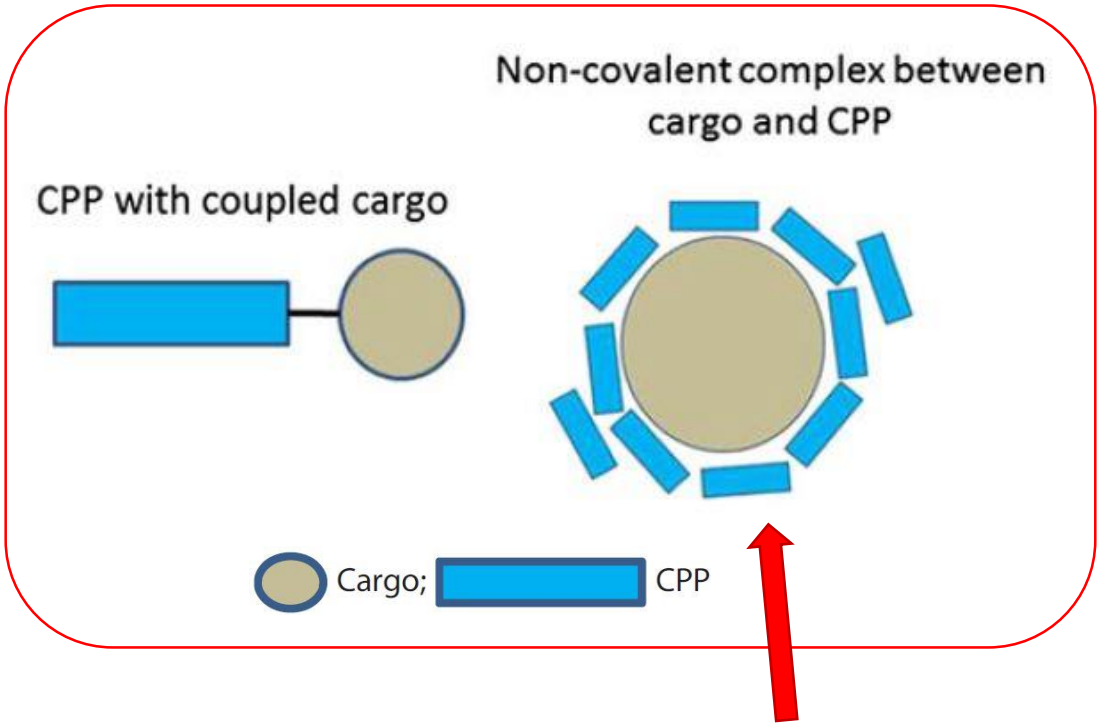


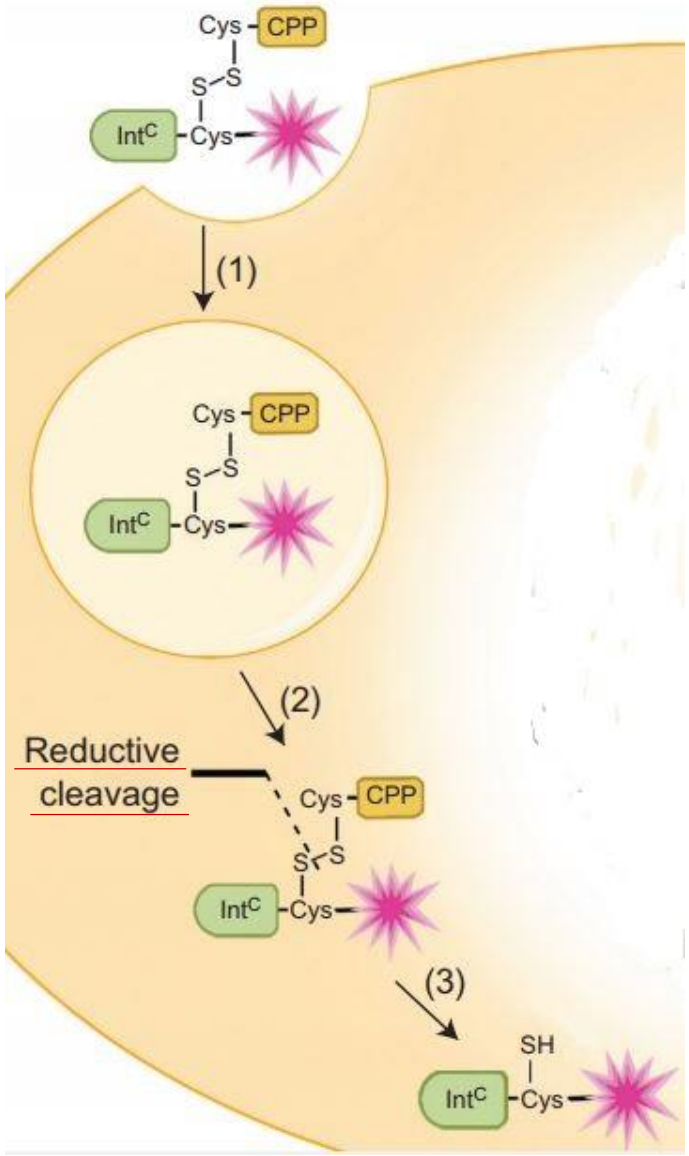
C105Y is a synthetic peptide (CSIPPEVKFNKPFVYLI) based on the amino acid sequence corresponding to residues 359–374 of α1-antitrypsin.

Ref) M. Rhee and P. Davis *J. Biol. Chem.* 2006, 281, 6, 1233-1240

3. Applications of CPPs

Assembly of complex CPP structures





CPP doesn't change the nature of cargo.

But, only a few CPPs are applicable to this method.

Amphipathic CPPs are likely to be applicable because of their hydrophobic interaction with cargo.

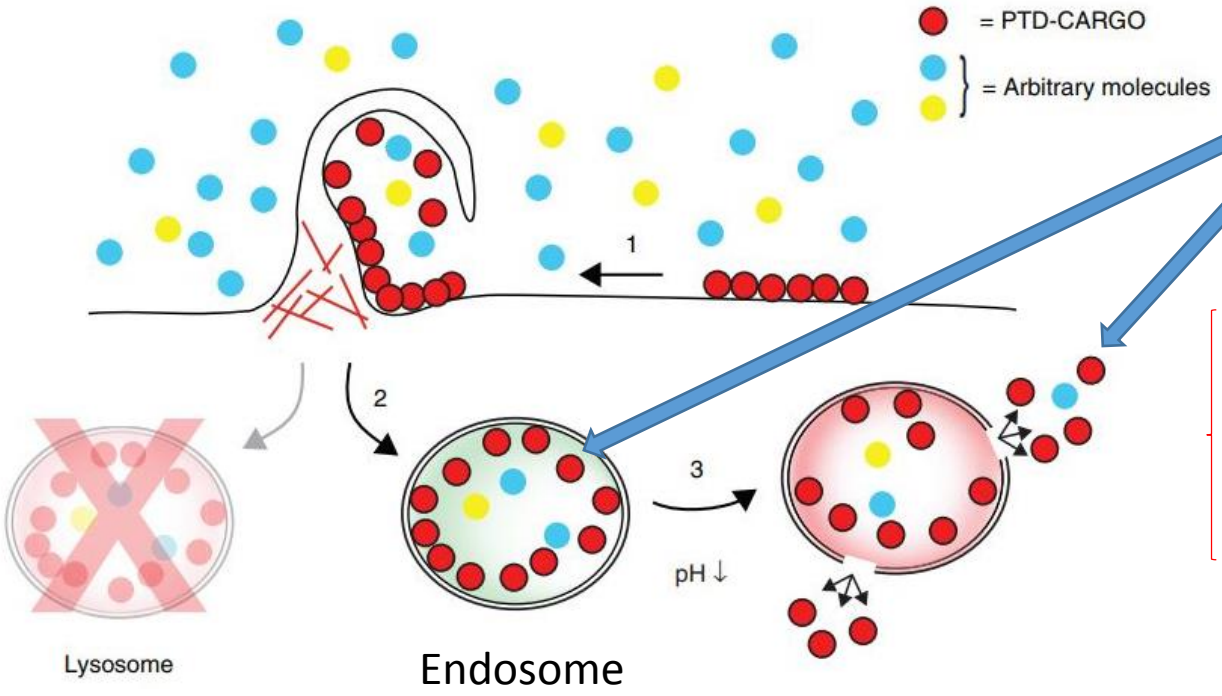
Ref) Siegmund Reissmann *J. Pept. Sci.* 2014, 20, 760–784

Comparison between CPPs

	Cationic	***Amphipathic***	***Hydrophobic***
Uptake mechanism	**Endocytosis**	**Direct insertion**	**Direct insertion**
Concentration tolerance	**High**	**Moderate**	**Low**
Applicability	**▪ Big size compounds (ex. antibody)** **▪ Some agents can help endosomal escape**	**▪ Acidically weak compounds** **▪ Non-covalent complex with cargo**	Unknown

※ Actually, which one is better depends on the nature of the compound and cell which you want to introduce.

Enhancing the metabolic stability of CPPs



Peptidase degrades CPP.

- The membrane permeation efficiency drops.
- The localization of cargo changes.

Stabilization of CPPs

① **Changing L-form amino acid to D-form**

② ***N*-methylation**

③ **Cyclization**

Ref) van den Berg, A. and Dowdy, S.F. *Curr. Opin. Biotechnol*. 2011, 22, 888–893

Ref) Siegmund Reissmann *J. Pept. Sci.* 2014, 20, 760–784

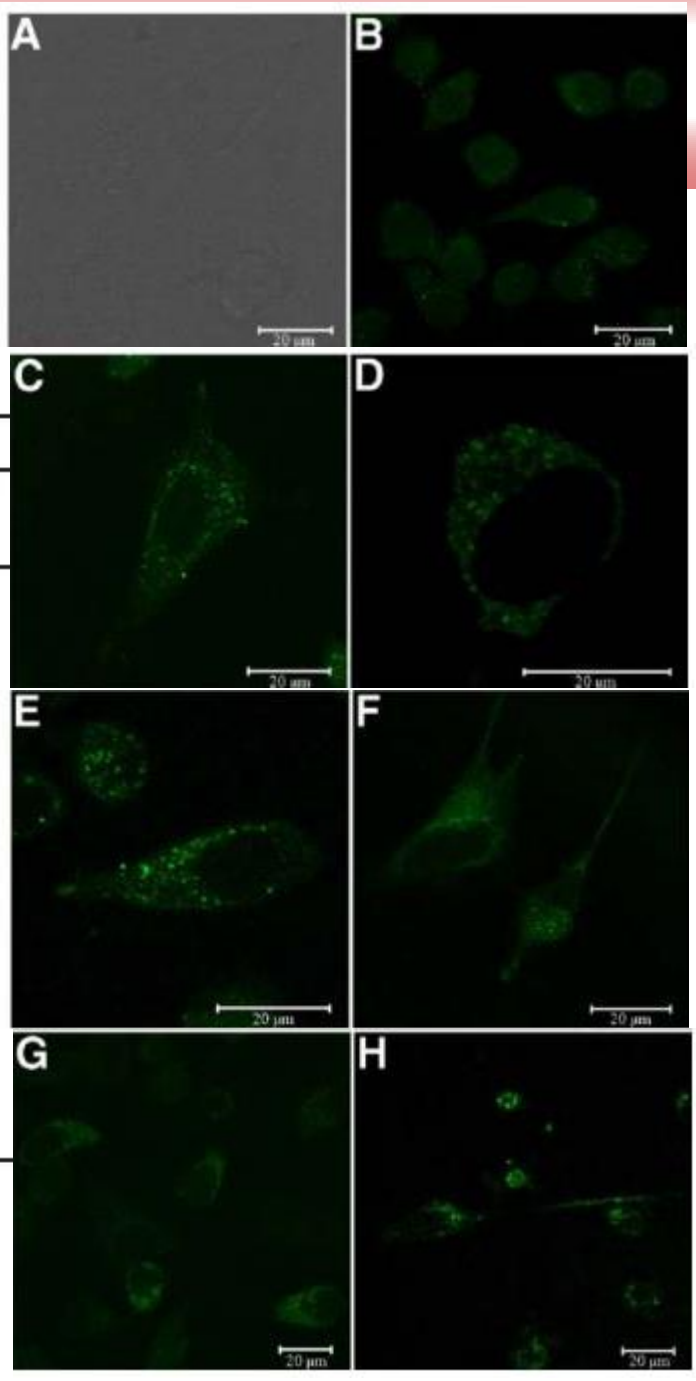
①Changing L-form amino acid to D-form
②*N*-methylation

hCT(9 – 32) : Amphipathic CPP

only CF (A)

Peptides Name and sequence		Half-life [h] human blood plasma
hCT(9–32) LGTYTQDFNKFHTFPQTAIGVGAP-NH_2	(B)	36.2±3.2
[f^{12}]-hCT(9–32) LGTfTQDFNKFHTFPQTAIGVGAP-NH_2	(C)	51.2±1.7
[f^{16}]-hCT(9–32) LGTYTQDfNKFHTFPQTAIGVGAP-NH_2	(D)	51.0±4.9
[f^{12,16}]-hCT(9–32) LGTfTQDfNKFHTFPQTAIGVGAP-NH_2	(E)	59.6±7.4
[*N*-Me-F^{12}]-hCT(9–32) LGT-***N*-Me-F**-TQDFNKFHTFPQTAIGVGAP-NH_2	(F)	37.2±4.9
[*N*-Me-F^{16}]-hCT(9–32) LGTYTQD-***N*-Me-F**-NKFHTFPQTAIGVGAP-NH_2	(G)	53.5±8.2
[*N*-Me-F^{12,16}]-hCT(9–32) LGT-***N*-Me-F**-TQD-***N*-Me-F**-NKFHTFPQTAIGVGAP-NH_2	(H)	126.2±15.9

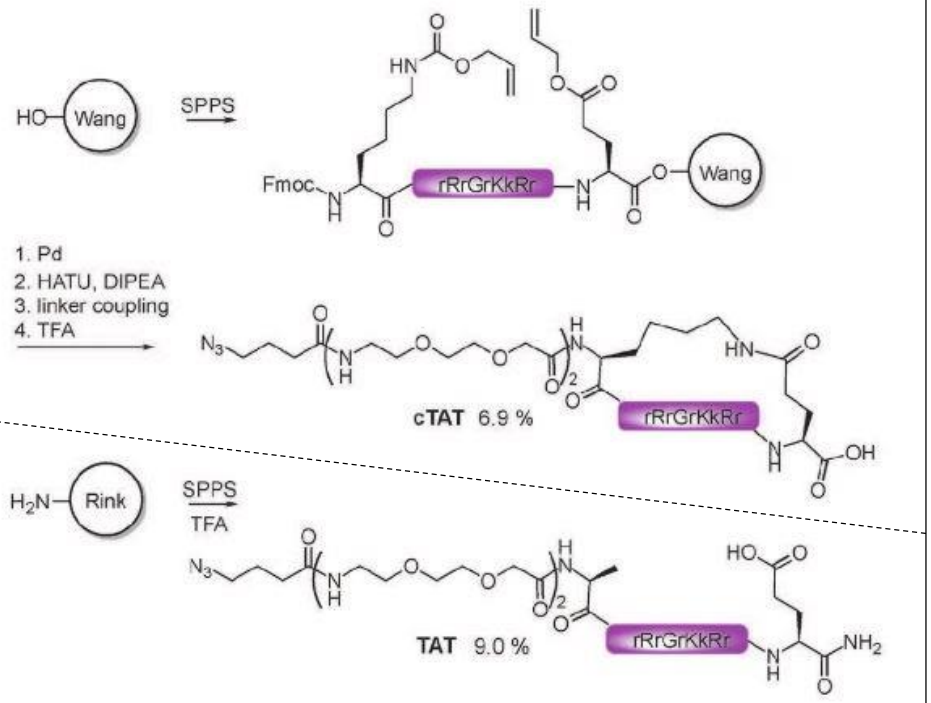
[a] All peptides are N-terminally labelled with CF.



Ref) R. Rennert et al. *Biochimica et Biophysica Acta* 2006, 1758, 347 – 354

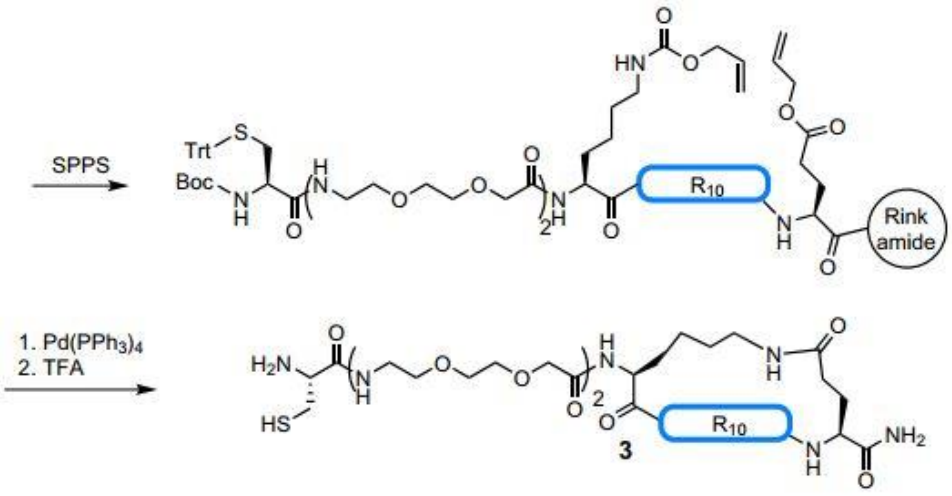
③ Cyclization

Cyclic TAT



Linear TAT

Cyclic R10



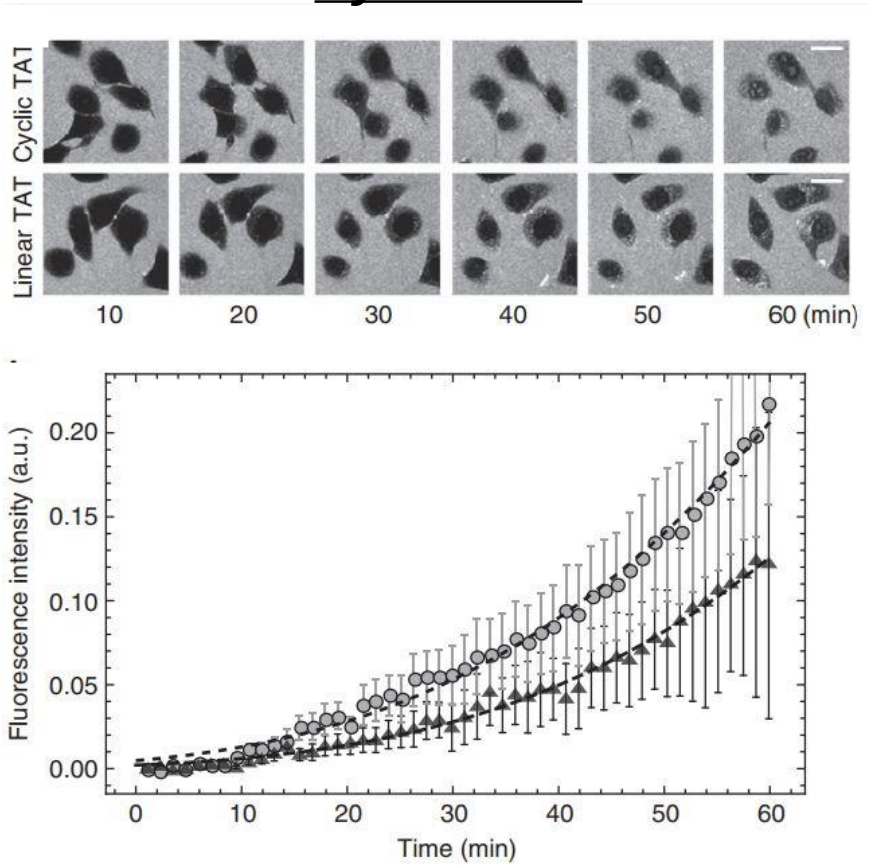
Ref) Cardoso C. et al. *Nat. Com.* 2011, 2, 453, 1–6

Ref) Cardoso C., Hackenberger R. et al. *Nat. Chem.* 2017, 9, 762–771

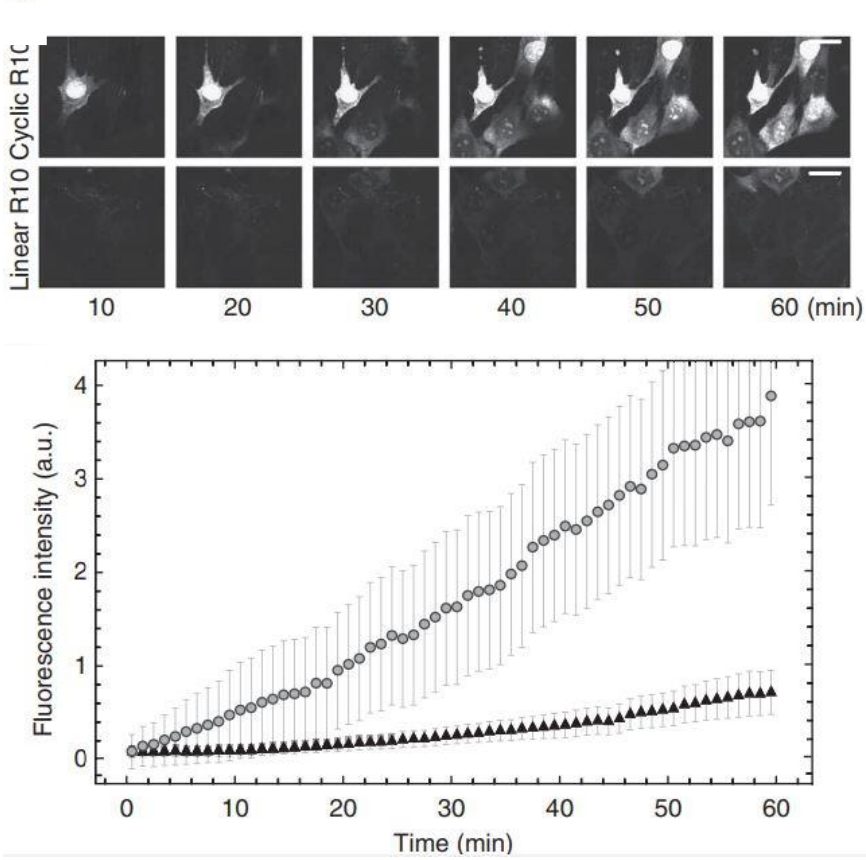
Ref) Cardoso C., Hackenberger R. et al. *Angew. Chem. Int. Ed.* 2015, 54, 1950 –1953

Cyclic TAT and R10

Cyclic TAT



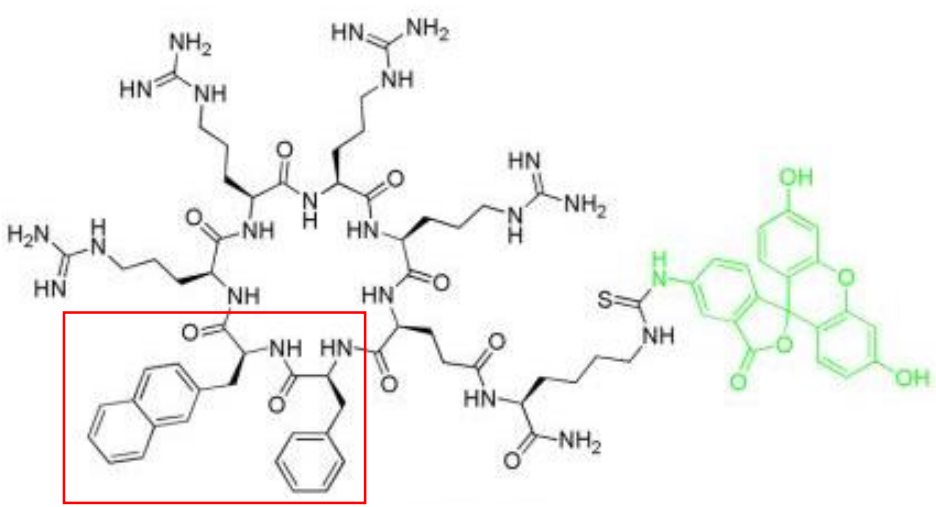
Cyclic R10



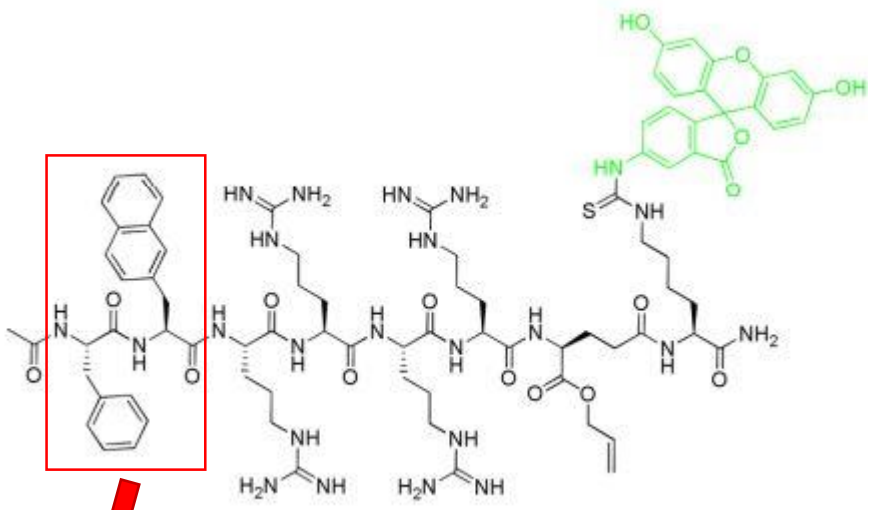
Cyclic CPPs have enhanced cell permeability than linear CPPs because of their metabolic stability.

Ref) Cardoso C. et al. *Nat. Com.* 2011, 2, 453, 1–6
Ref) Cardoso C., Hackenberger R. et al. *Nat. Chem.* 2017, 9, 762–771
Ref) Cardoso C., Hackenberger R. et al. *Angew. Chem. Int. Ed.* 2015, 54, 1950 –1953

Cyclic Amphipathic CPP



cFΦR$_4$-FITC



FΦR$_4$-FITC

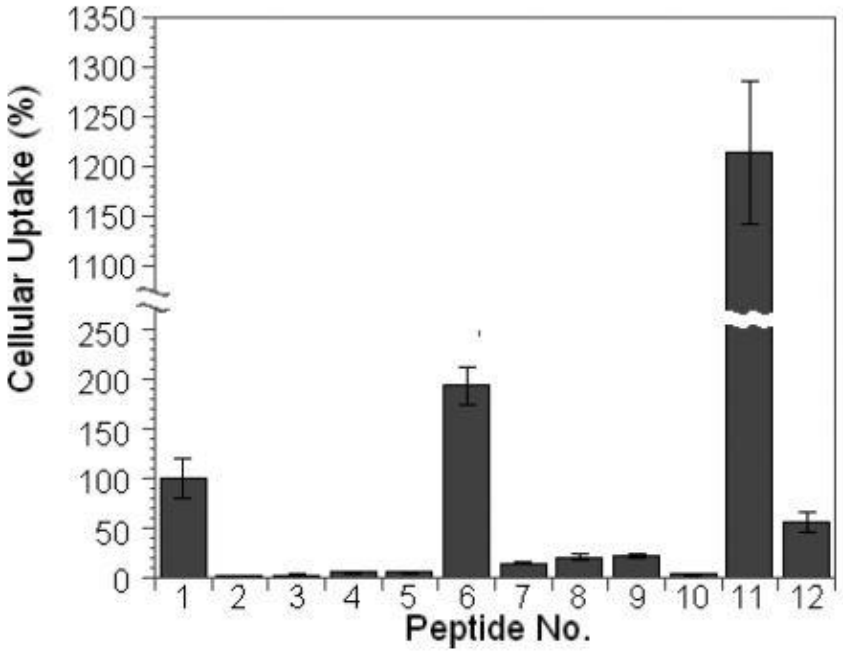
F and Φ(L-2-naphthylalanine) enhances the membrane transduction activity of CPPs.

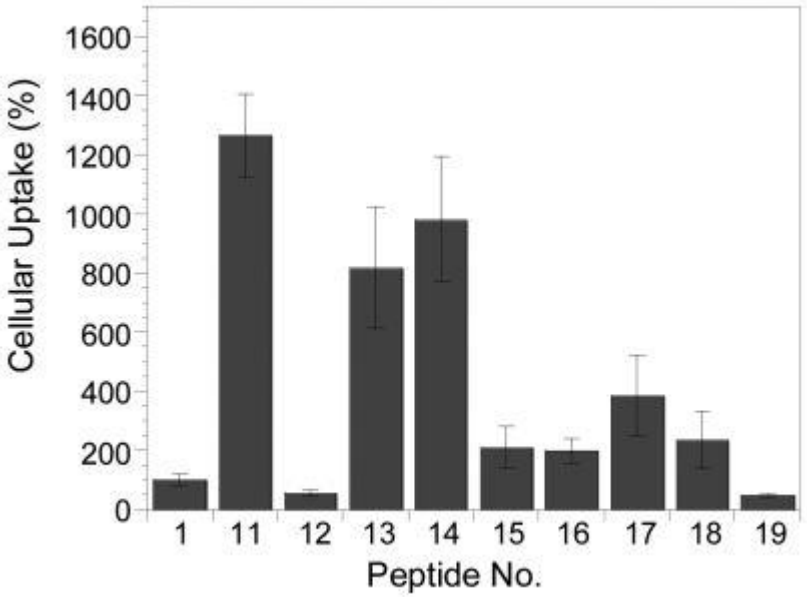
Ref) Dehua Pei et al. *ACS Chem. Biol*. 2013, 8, 423−431

Ref) Dehua Pei et al. Biochemistry 2014, 53, 4034−4046

Cyclic Amphipathic CPP

peptide no.	abbreviation	peptide sequence[b]	cellular association (%)[c]
1	R_9	Ac-RRRRRRRRRQ-OAll	100
2	cR_4	cyclo(RRRRQ)	2.5 ± 0.5
3	R_4	Ac-RRRRQ-OAll	2.3 ± 0.8
4	cR_6	cyclo(RRRRRRQ)	5.8 ± 1.1
5	R_6	Ac-RRRRRRQ-OAll	5.8 ± 1.0
6	cF_2R_4	cyclo(FFRRRRQ)	190 ± 20
7	F_2R_4	Ac-FFRRRRQ-OAll	14 ± 1
8	cr_6	cyclo(rrrrrrQ)	21 ± 3
9	r_6	Ac-rrrrrrQ-OAll	21 ± 2
10	cA_4R_4	cyclo(AAAARRRRQ)	3.2 ± 0.2
11	$cF\Phi R_4$	cyclo(FΦRRRRQ)	1260 ± 140
12	$F\Phi R_4$	Ac-FΦRRRRQ-OAll	55 ± 10
13	$cAF\Phi R_4$	cyclo(AFΦRRRRQ)	820 ± 210
14	$cA_2F\Phi R_4$	cyclo(AAFΦRRRRQ)	980 ± 210
15	$cA_3F\Phi R_4$	cyclo(AAAFΦRRRRQ)	210 ± 70
16	$cA_4F\Phi R_4$	cyclo(AAAAFΦRRRRQ)	200 ± 40
17	$cA_5F\Phi R_4$	cyclo(AAAAAFΦRRRRQ)	390 ± 140
18	$cA_6F\Phi R_4$	cyclo(AAAAAAFΦRRRRQ)	240 ± 100
19	$cA_7F\Phi R_4$	cyclo(AAAAAAAFΦRRRRQ)	47 ± 5

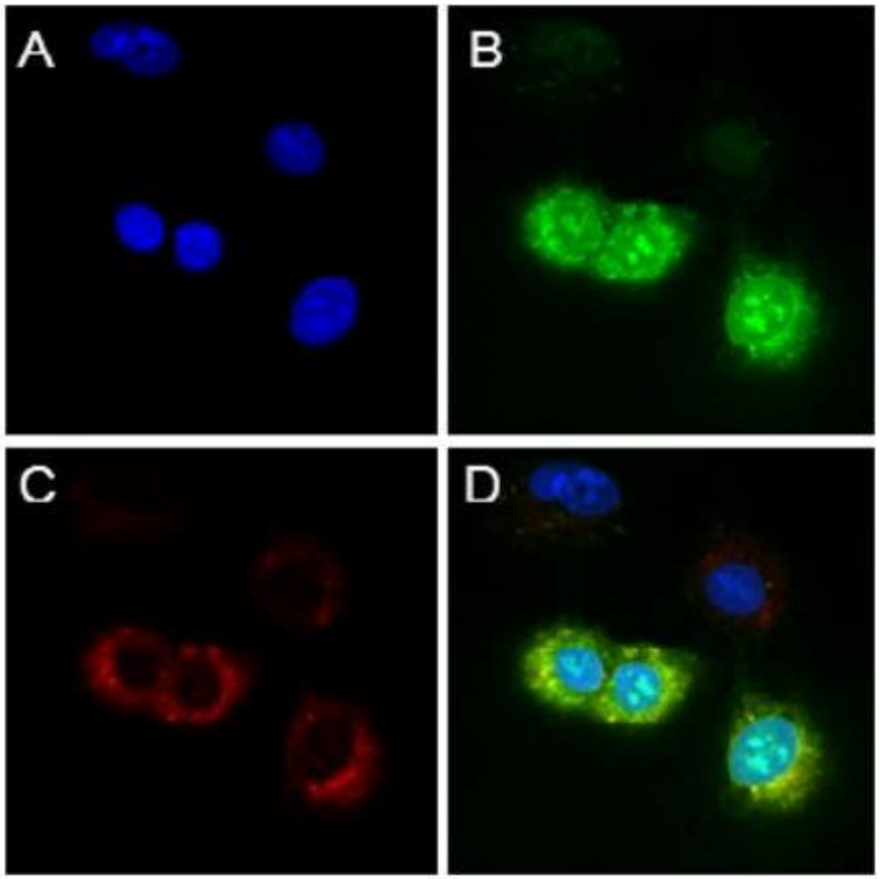




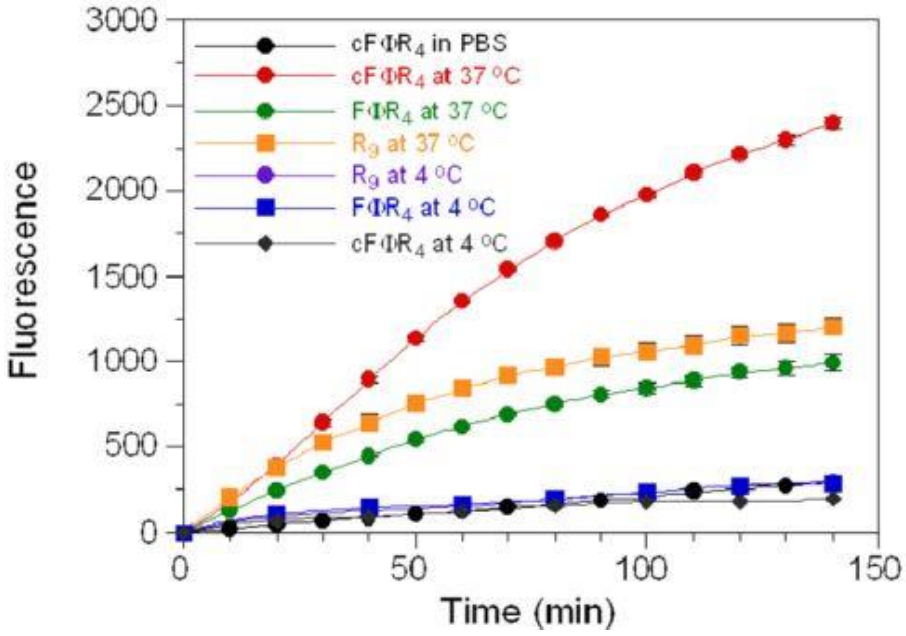
Ref) Dehua Pei et al. *ACS Chem. Biol*. 2013, 8, 423−431

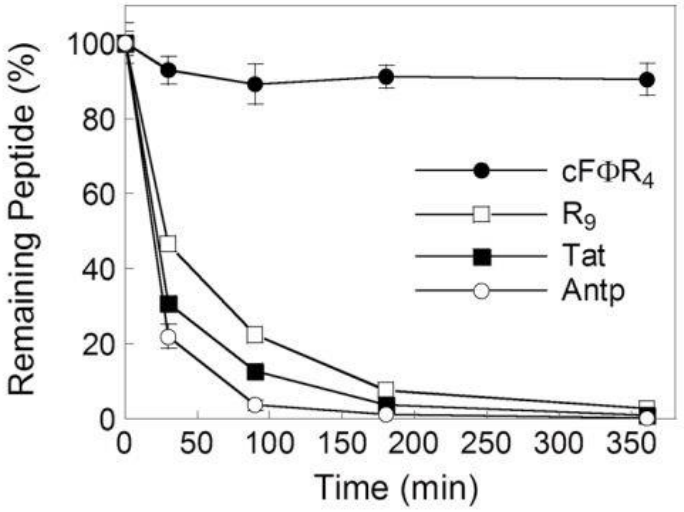
Ref) Dehua Pei et al. Biochemistry 2014, 53, 4034−4046

Cyclic Amphipathic CPP



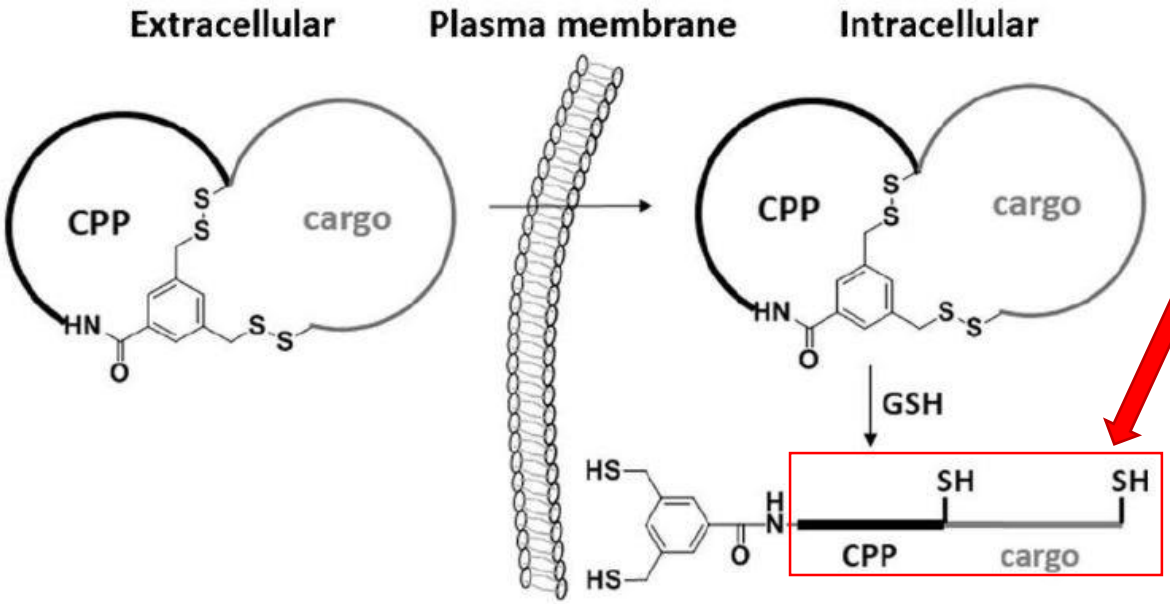
(A) Nuclear stain with DRAQ5
(B) Green fluorescence of internalized cFΦR4-FITC
(C) Red fluorescence of rhodamine B-dextran
(D) A merge of panels A−C





Ref) Dehua Pei et al. *ACS Chem. Biol*. 2013, 8, 423−431
Ref) Dehua Pei et al. Biochemistry 2014, 53, 4034−4046

Bicyclization



CPP and cargo, which were cyclic, regain the linear character in cell.

Many peptide ligands must be in their linear conformations to be biologically active.

Peptide ID	Sequence
1	RQIKIWFQNRRMKWKKGG-TALDWSWLQTE
4	BMB-RRRRΦF-C-ALDWSWLQ-C
5	BMB-RRRRΦF-C-ALDASALQ-C
6	MP-RRRRΦF-C-ALDWSWLQ

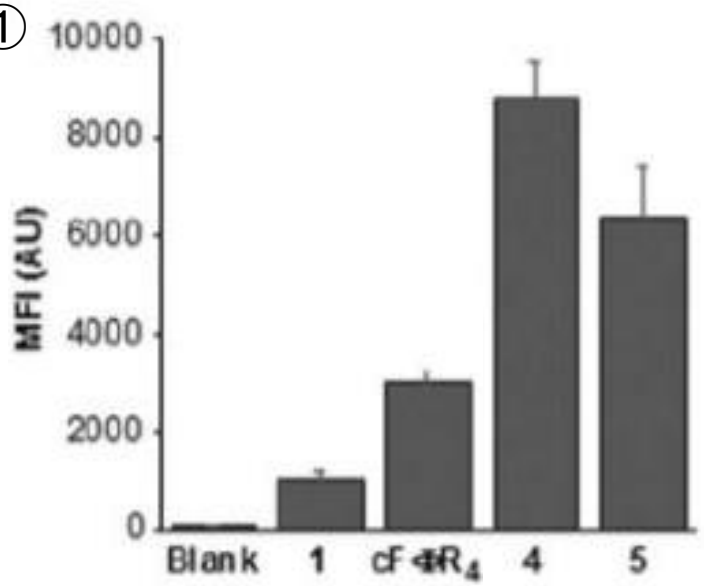
NEMO-binding domain (NBD) reduces aberrant NF-κB activity.

NF-κB is a transcription factor that controls the expression of numerous gene products.

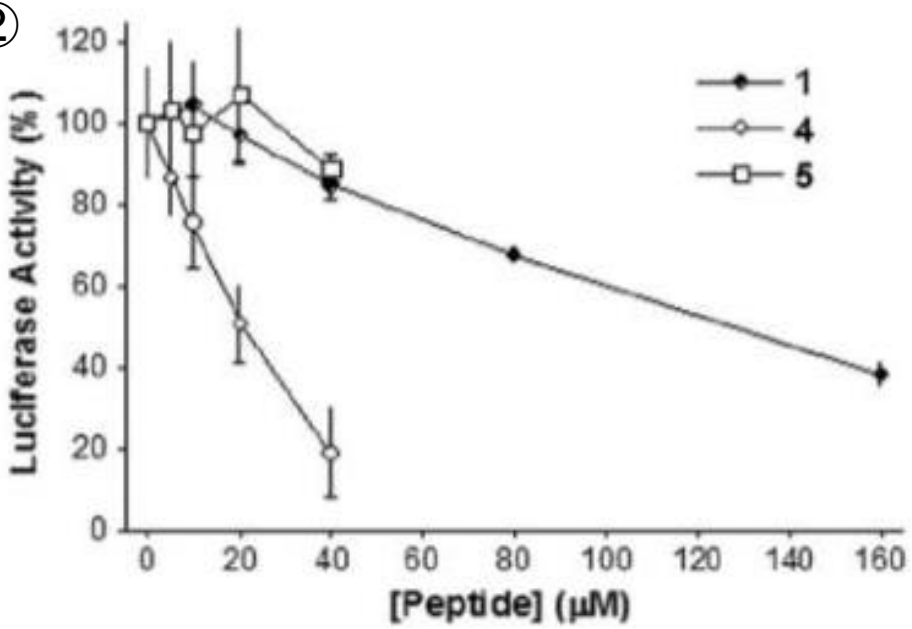
Ref) Dehua Pei et al. *Angew.Chem. Int.Ed.* 2017, 56,1525 –1529

Bicyclization

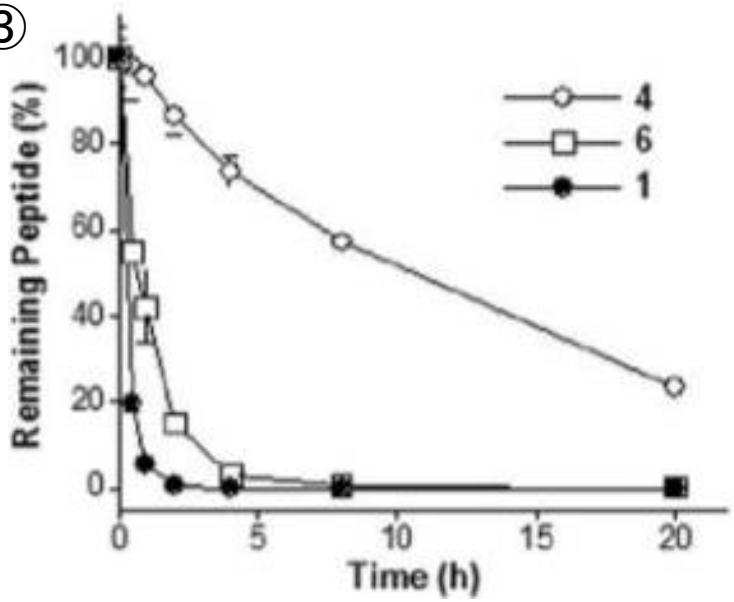
①



②



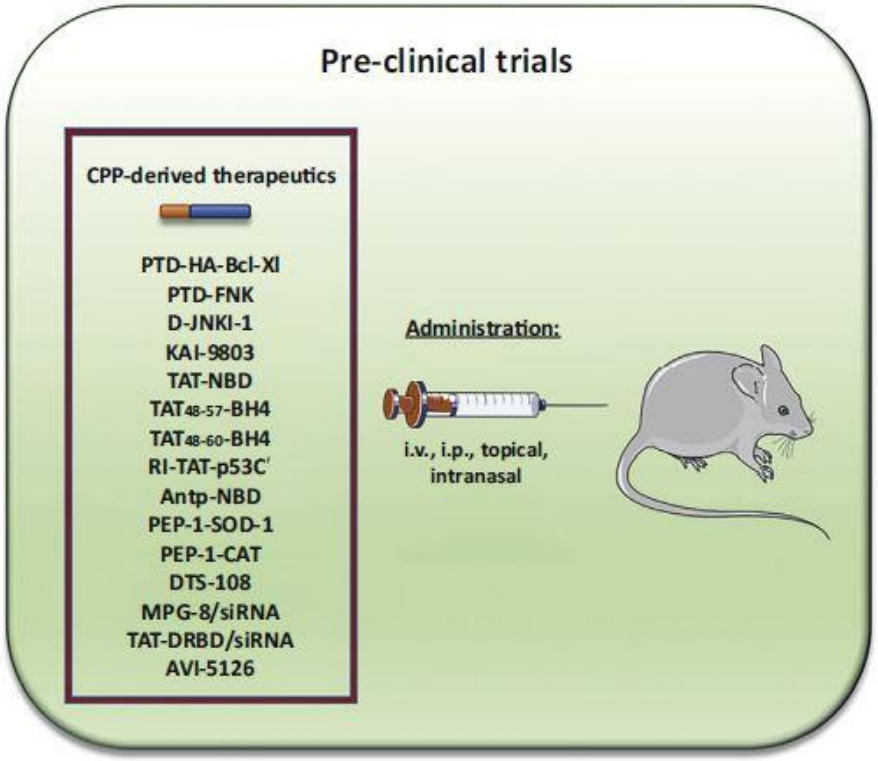
③

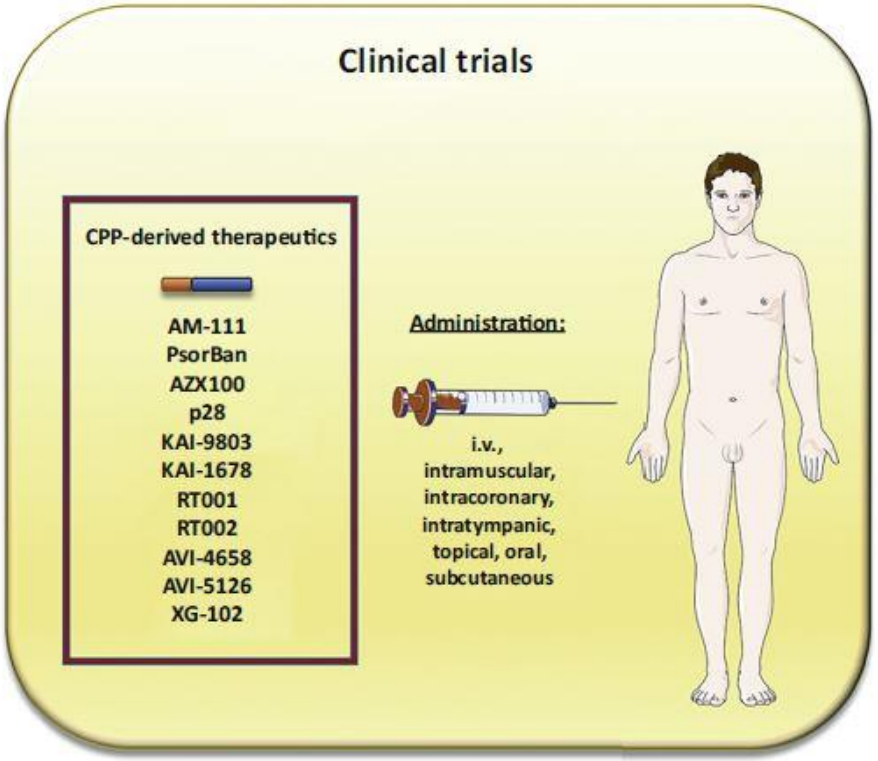


① : Peptides were labeled with fluorescein FITC on the sidechain of a C-terminal lysine. Mean fluorescence intensity (MFI) values showed their cellular uptake.
② : Cells transfected with a luciferase reporter gene under the control of NF-κB were first treated with varying concentrations of a peptide and then TNFα. TNFα activates NF-κB.
③ : The metabolic stability of peptides was tested by incubating the peptides in human serum.

Ref) Dehua Pei et al. *Angew.Chem. Int.Ed.* 2017, 56,1525 –1529

Application for Preclinical and Clinical Evaluations





Ref) Daniela Rossi et al. *Trends in Pharmacological Sciences* 2017, 38, 406-424

Amphipathic CPPs interact with lipids

P294 : Ac-GALFL GFLGAAGSTM GAWSQ PKKKR KV-Cya

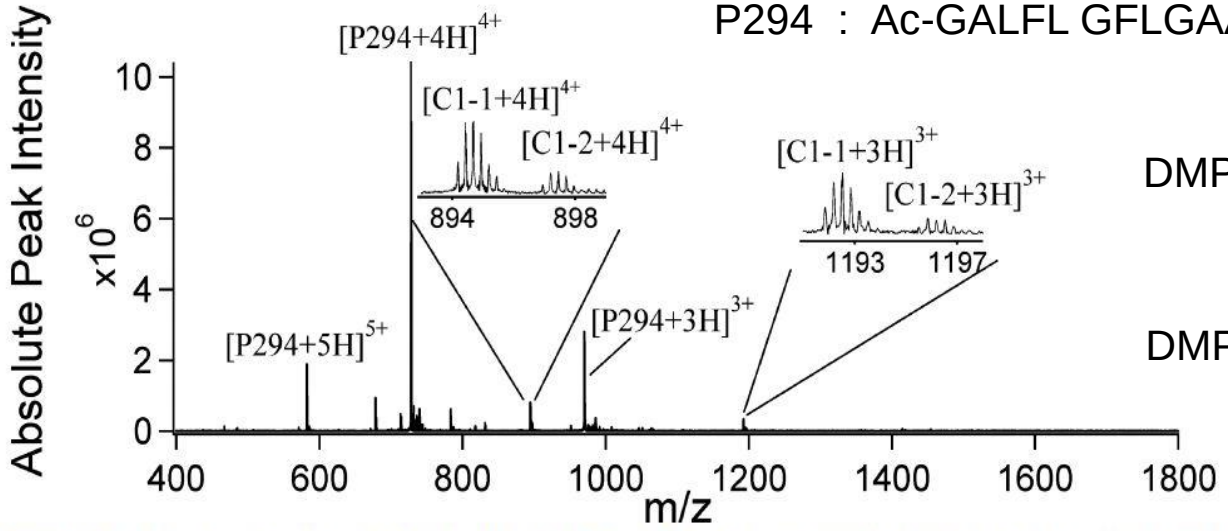
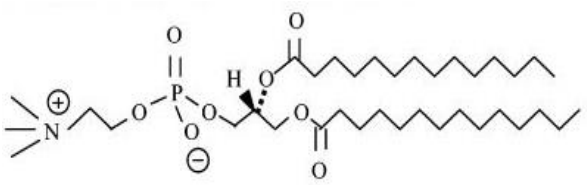
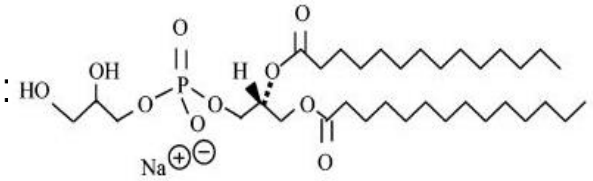


Figure 1. Electrospray mass spectrum of 20:20:20 μM P294–DMPC–DMPG in aqueous solution. "C1-1" and "C1-2" represent [P294 + DMPG − Na + H] and [P294 + DMPC] complexes, respectively.

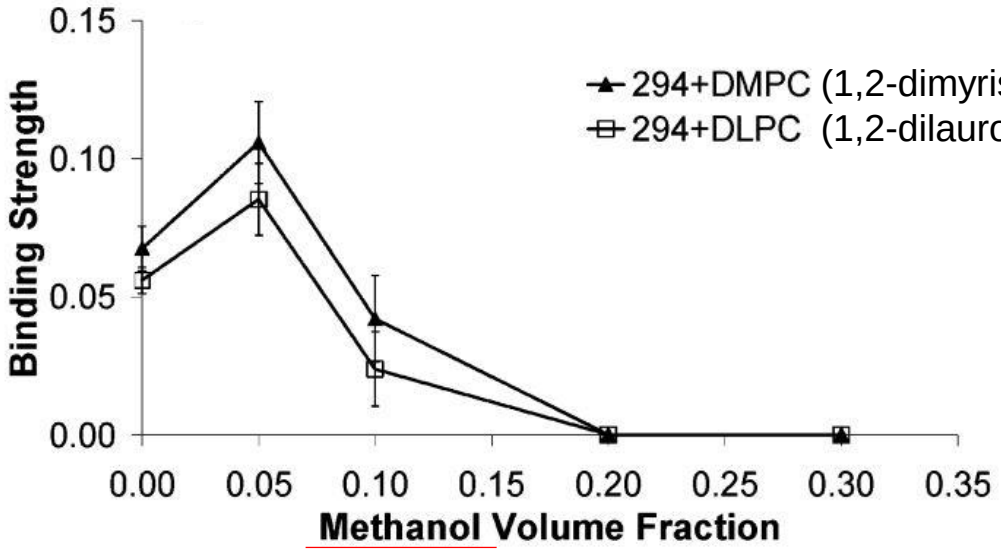
DMPC :



DMPG :



C1-1 : [P294 + DMPG]
C1-2 : [P294 + DMPC]



P294 forms stable 1:1 complexes with lipids. And hydrophobic interaction is essential for forming complex.

weakens the hydrophobic interactions

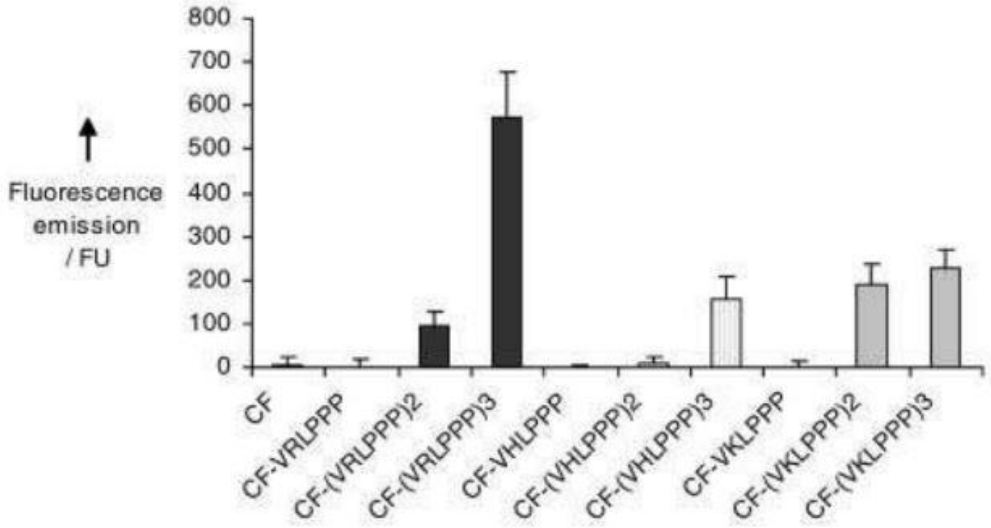
Ref) Richard B. Cole et al. *Anal. Chem*. 2005, 77, 1556-1565

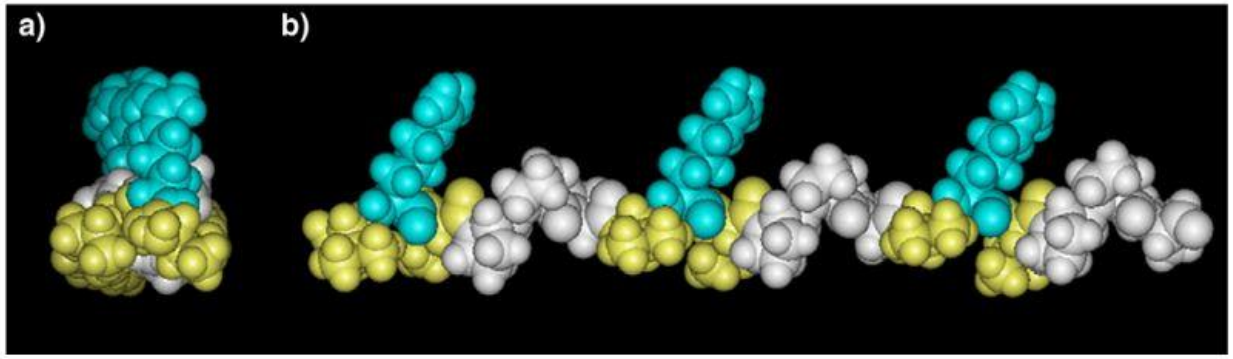
Proline-rich CPPs

It was discovered that …

- N-terminal γ-zein domain's octamer $(VHLPPP)_8$ can interact with cell membranes.
- A peptide containing just proline residues P_{14} can internalize in cells.

Pro content of 50% is enough to maintain a helical structure.



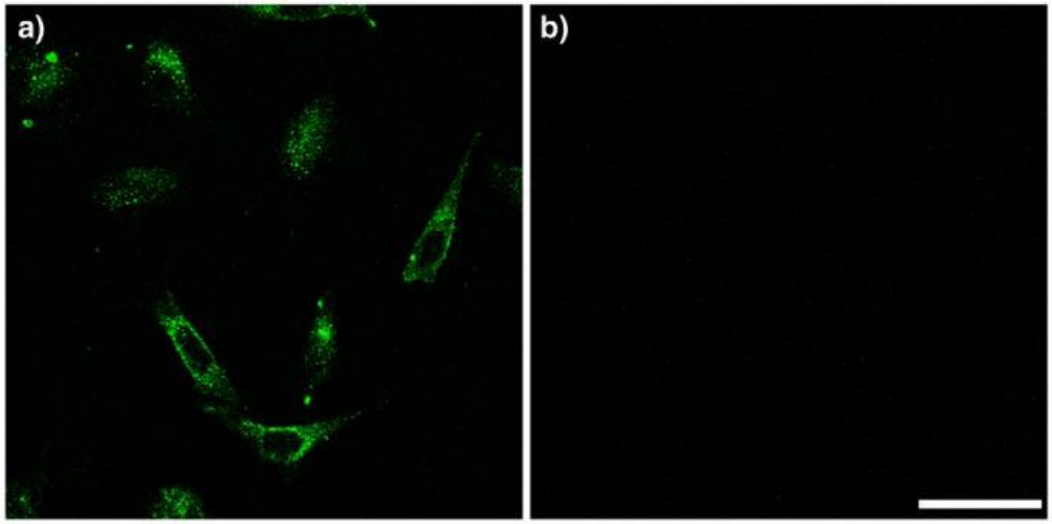


Ref) J. Fernandez-Carneado, M.J. Kogan, S. Castel, E. Giralt, *Angew. Chem. Int. Ed. Engl.* 2004, 43, 1811–1814

Ref) Pujals, S. and Giralt, E. *Adv. Drug Deliv. Rev*. 2008, 60, 473–484

Proline-rich CPPs

CF-(VRLPPP)$_8$



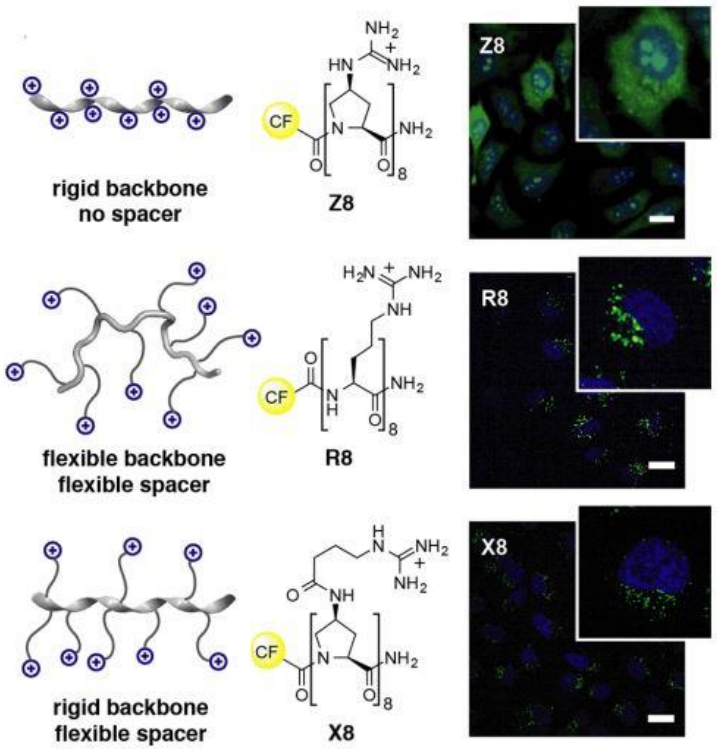
@ 37 ℃ **@ 4 ℃**

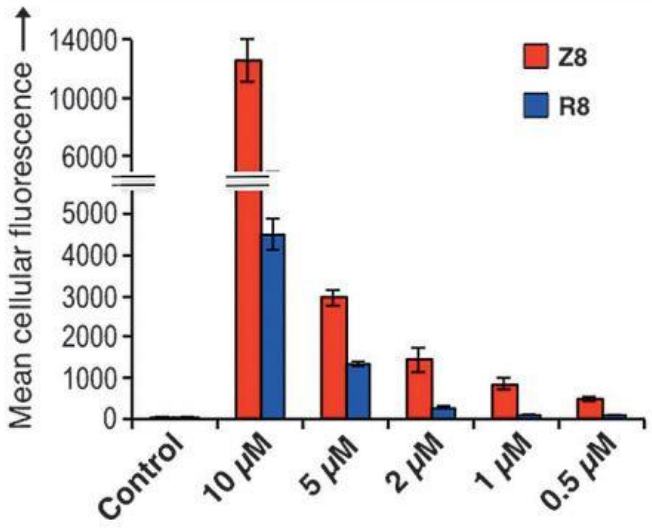
CF-(VRLPPP)$_8$ internalize in cell by endocytosis.

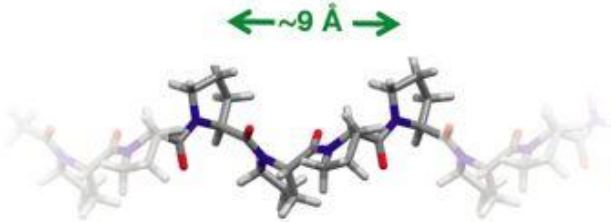
Ref) J. Fernandez-Carneado, M.J. Kogan, S. Castel, E. Giralt, *Angew. Chem. Int. Ed. Engl.* 2004, 43, 1811–1814

Ref) Pujals, S. and Giralt, E. *Adv. Drug Deliv. Rev*. 2008, 60, 473–484

Poly-proline as CPP

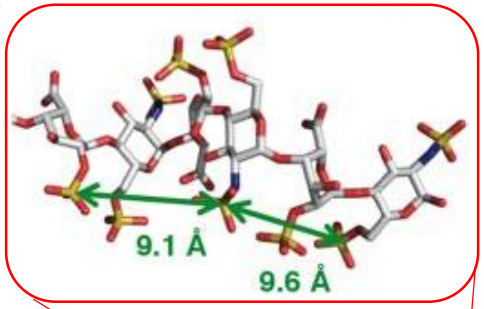




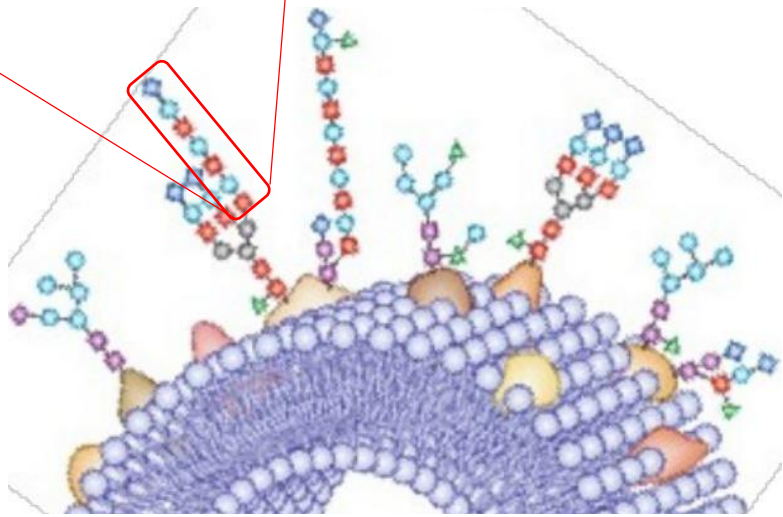


Poly-proline

Good interaction

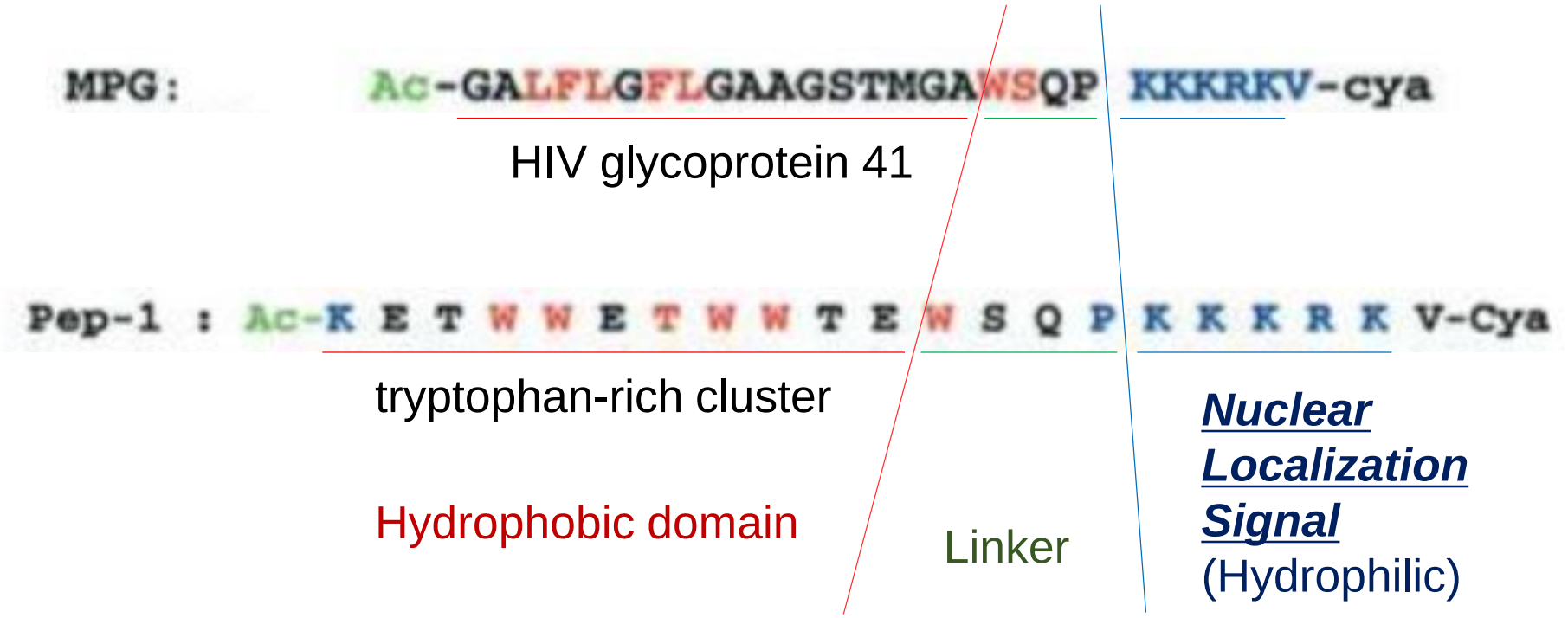


Anionic cell-surface glycan



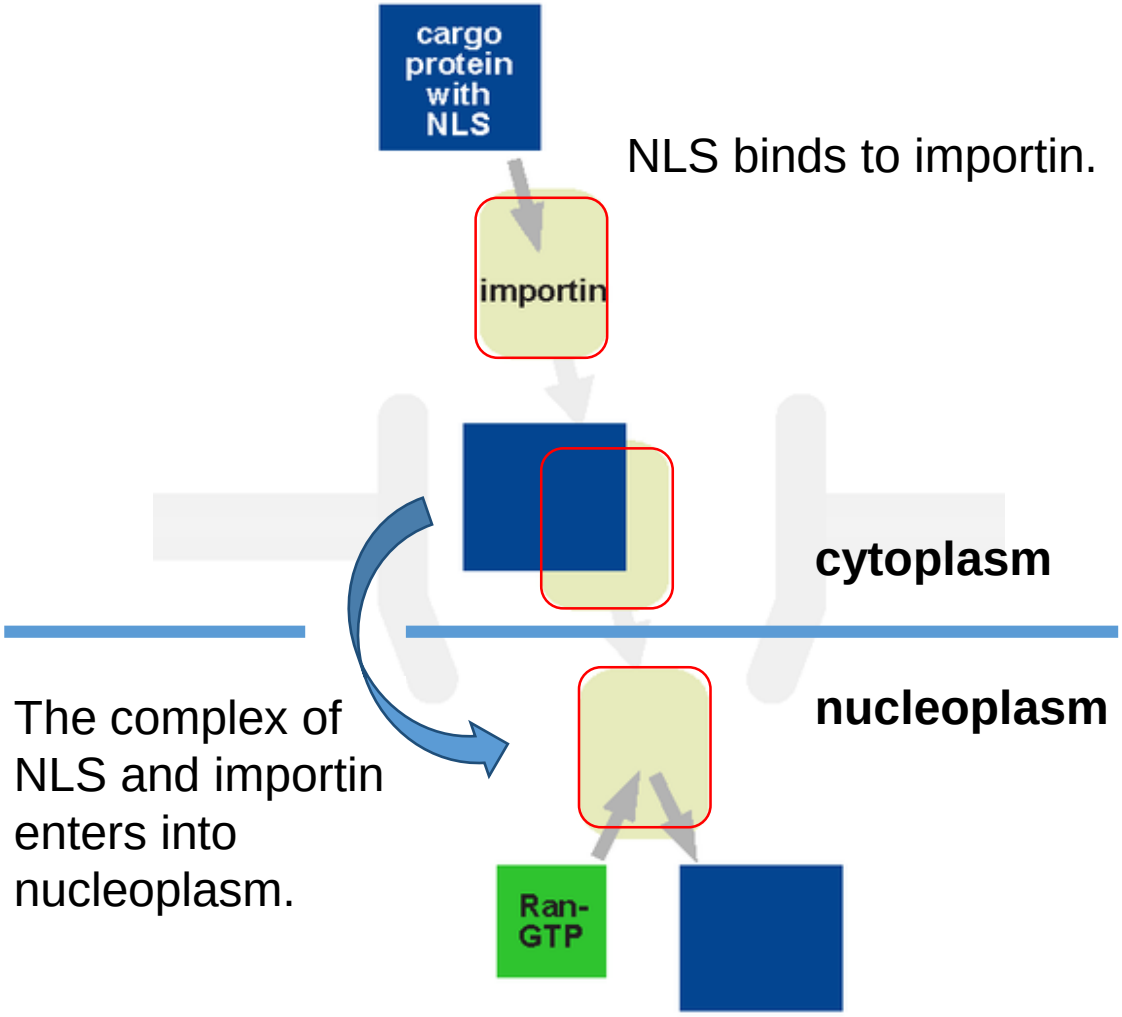
Ref) H. Wennemers et al. *Angew.Chem.Int. Ed*. 2017, 56,122 –126

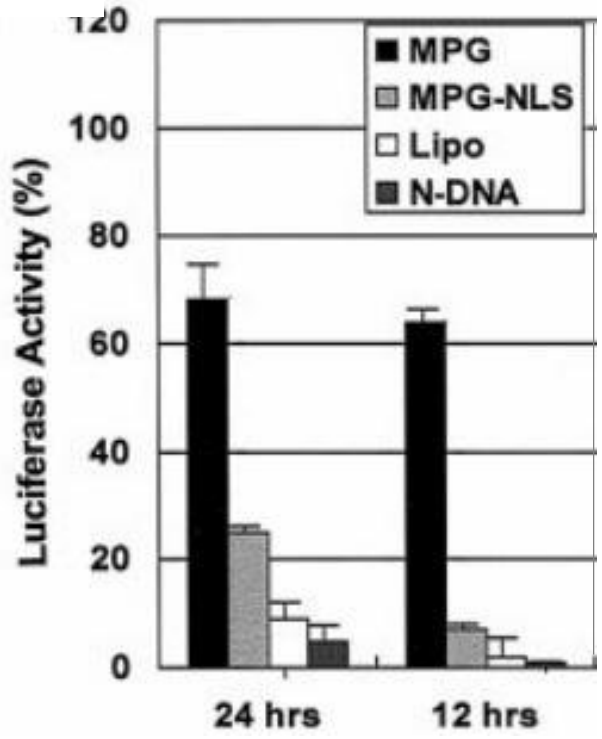
MPG and Pep-1



Ref) Morris, M.C. et al. *Biol. Cell* 2008, 100, 201–217
Ref) Gilles Divita et al. *Nuc. Acid. Res.* 2003, 31, 11, 2717-2724

Nuclear Localization Signal





The NLS of MPG is essential for nuclear translocation.

Ref) Morris, M.C. et al. *Biol. Cell* 2008, 100, 201–217
Ref) Gilles Divita et al. *Nuc. Acid. Res.* 2003, 31, 11, 2717-2724