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FACULTY OF **PHARMACEUTICAL SCIENCES**

HOW THE EXPLORATION OF THE CHEMICAL SPACE OF CELL-PENETRATING PEPTIDES HELPS TO UNDERSTAND THEIR FUNCTIONALITY

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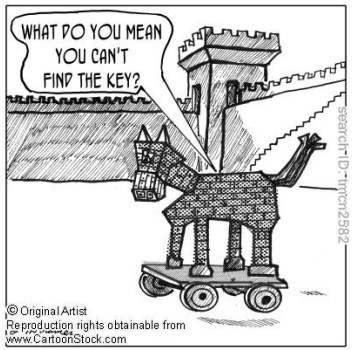
INTRODUCTION TO CELL-PENETRATING PEPTIDES (CPPs)

- Discovered about 20 years ago.
- "Short, cationic peptides (5-30 AA)"
  → Chemically diverse group of peptides
- Cross cell membrane barriers.
  → No significant membrane damage.
- Carriers for cell membrane impermeable cargoes.
  e.g. small molecules, oligonucleotides, peptides and proteins.
- Also biological effects.
  e.g. antimicrobial activity.



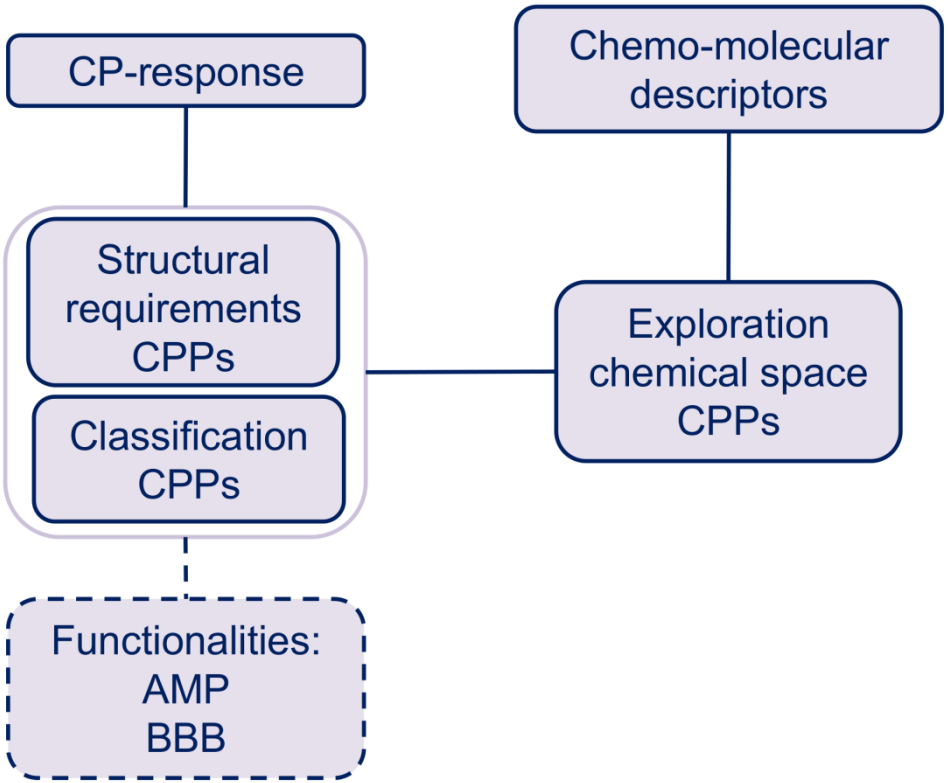
QUANTITATIVE DATA FOR CELLULAR INFLUX OF CPPs ARE NOT COMPARABLE

- Database of 186 CPPs (PLOS ONE, 8(8), e71752).
- Variety of techniques and experimental protocols.

Operational parameter	n	Examples
Technique	10	FACS, spectrofluorometry
Positive control	12	Penetratin, Tat 47-57
Negative control	9	No, green fluorescent peptide
Units of quantitative data	17	µM, a.u.
Label	12	FITC, 5,6-carboxyfluorescein
Cell line	53	HeLa, Jurkat
Incubation concentration	33	10nM – 1.6 mM

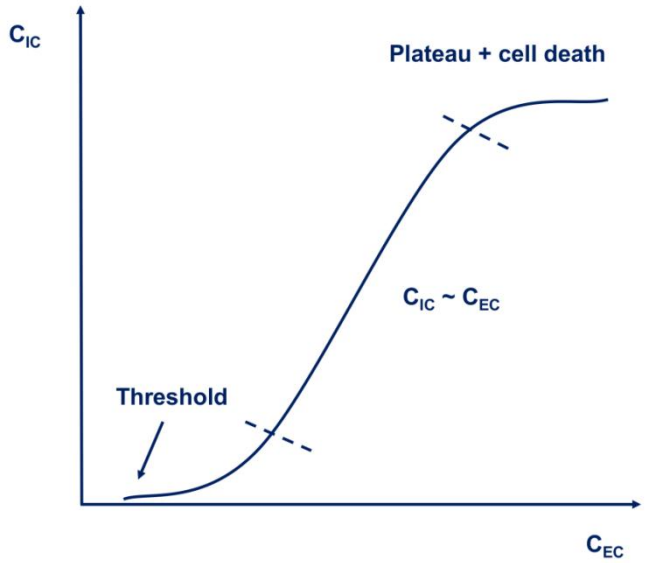
Mechanistic and structural properties of CPPs controversial

A UNIFIED RESPONSE FOR CELL-PENETRATING PROPERTIES OF PEPTIDES IS USED TO FIND CORRELATIONS WITH THEIR FUNCTIONALITIES



FIVE ASSUMPTIONS WERE MADE TO DEFINE THE CP-RESPONSE

1. Cell and label differences neglected.
2. Uptake negative control = negligible.
3. Maximal values cellular uptake used.
4. Multiple peptides → positive control selected.
5. $C_{intracelluar} \sim C_{extracellular}$



CP-RESPONSE = CELLULAR UPTAKE DATA NORMALIZED FOR CONCENTRATION AND POSITIVE CONTROL PENETRATIN

- **CP-response**: $$\frac{P_{CPP/}\,C_{CPP}}{P_{penetratin/}\,C_{penetratin}}$$ Range: 0.0014 – 2.74

E.g. Tat 48-60 (0.22) < R9 = penetratin (1.00) < pVEC (1.32) < transportan 10 (1.64) < MAP (1.72)

- *Positive control (PC) different from penetratin used:*

CP-response: $$\frac{P_{CPP}/C_{CPP}}{P_{PC}/C_{PC}} \times \text{response factor}$$

→ response factor = median CP-responses PC *e.g.* Tat 48-60 = 0.22

- *No positive control available*

→ Normalization: median CP-response penetratin ~ used technique

CP-RESPONSE = CELLULAR UPTAKE DATA NORMALIZED FOR CONCENTRATION AND POSITIVE CONTROL PENETRATIN

- **CP-response**:

$$\frac{P_{CPP}/C_{CPP}}{P_{penetratin}/C_{penetratin}}$$

E.g. calculation CP-response Tat 48-60 (Sugita *et al*., Br. J. Pharmacol. 153(6), 1143-1152)

Peptide	Cellular uptake response	Incubation concentration
Tat 48-60	1757 a.u.	10 µM
Penetratin	2162 a.u.	10 µM

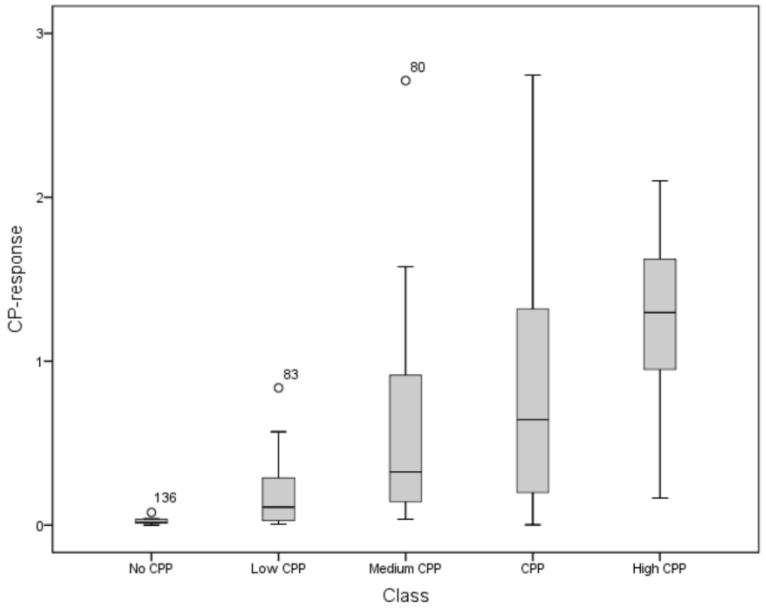
CP-response Tat 48-60 = $\frac{1757\ \text{a.u.}/10\ \mu\text{M}}{2162\ \text{a.u.}/10\ \mu\text{M}} = 0.81$

CALCULATED CP-RESPONSES FOR PEPTIDES CORRESPOND WITH THE APPRECIATION OF CELLULAR INFLUX BY THE AUTHORS

- Ranking of CPPs based on CP-response corresponds roughly with literature.

  Tat 48-60 (0.22) < R9 = penetratin (1.00) < pVEC (1.32) < transportan 10 (1.64) < MAP (1.72)

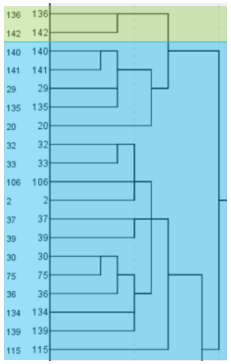
- Correspondence with appreciation by authors:



MULTIVARIATE DATA-ANALYSIS OF CHEMO-MOLECULAR DESCRIPTORS OF CPPs RESULTS IN SIX MAIN CLUSTERS AND EIGHT SUBCLUSTERS

Chemo-molecular descriptors → Principal component and hierarchical cluster analysis

HCA



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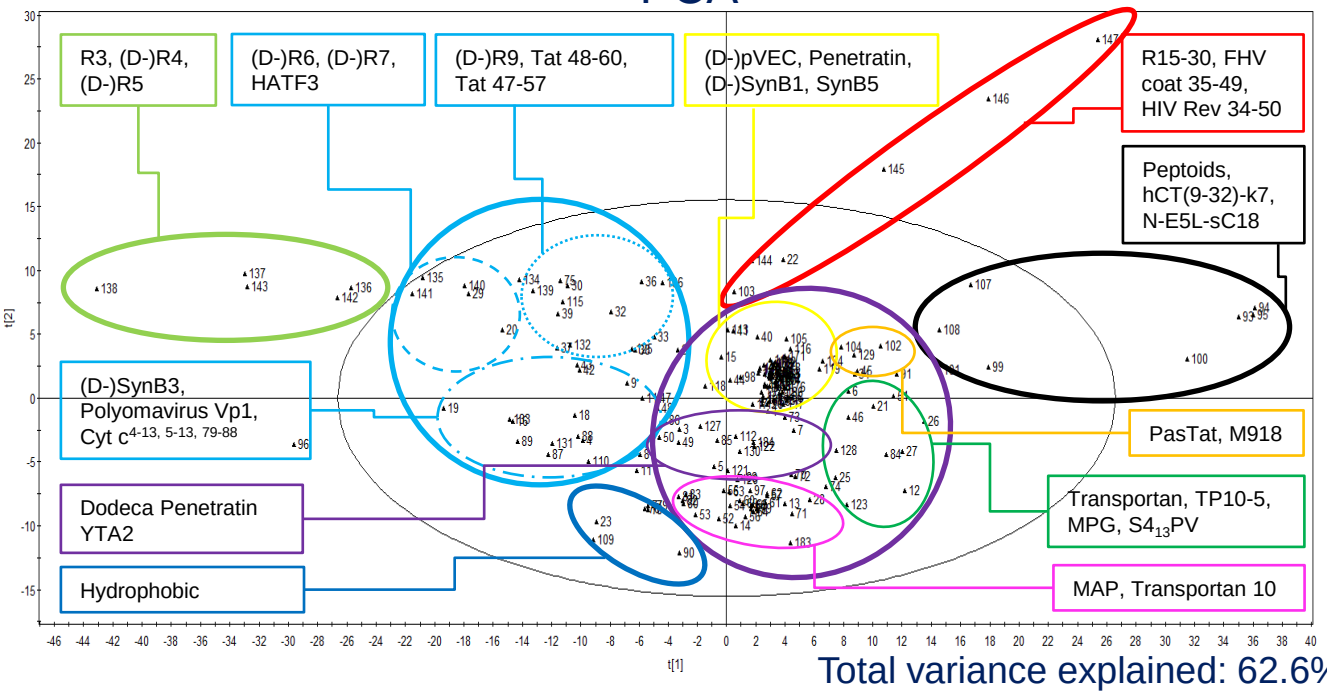
PCA



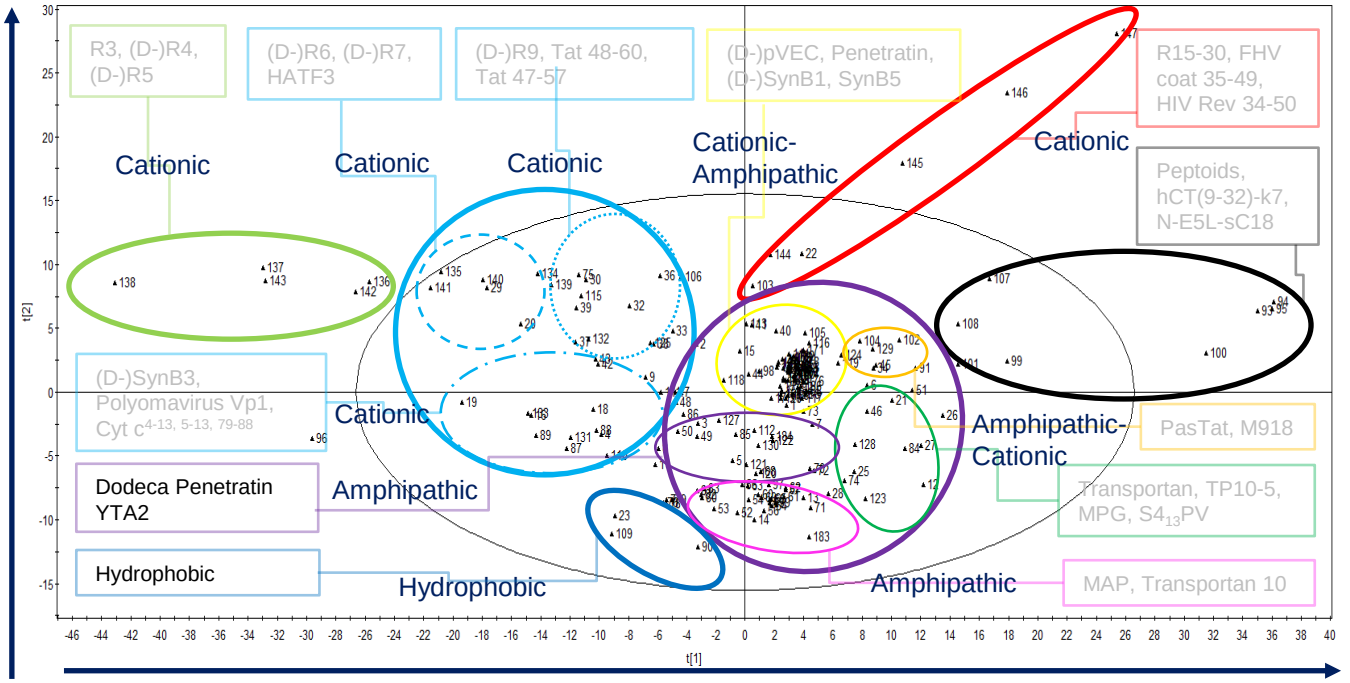
Total variance explained: 62.6%

CPPs ARE A CHEMICALLY DIVERSE GROUP OF PEPTIDES

Chemical

Functional

- Hydrophilic
- High charge density



- Hydrophobic

- Low MW
- Symmetric
- Compact

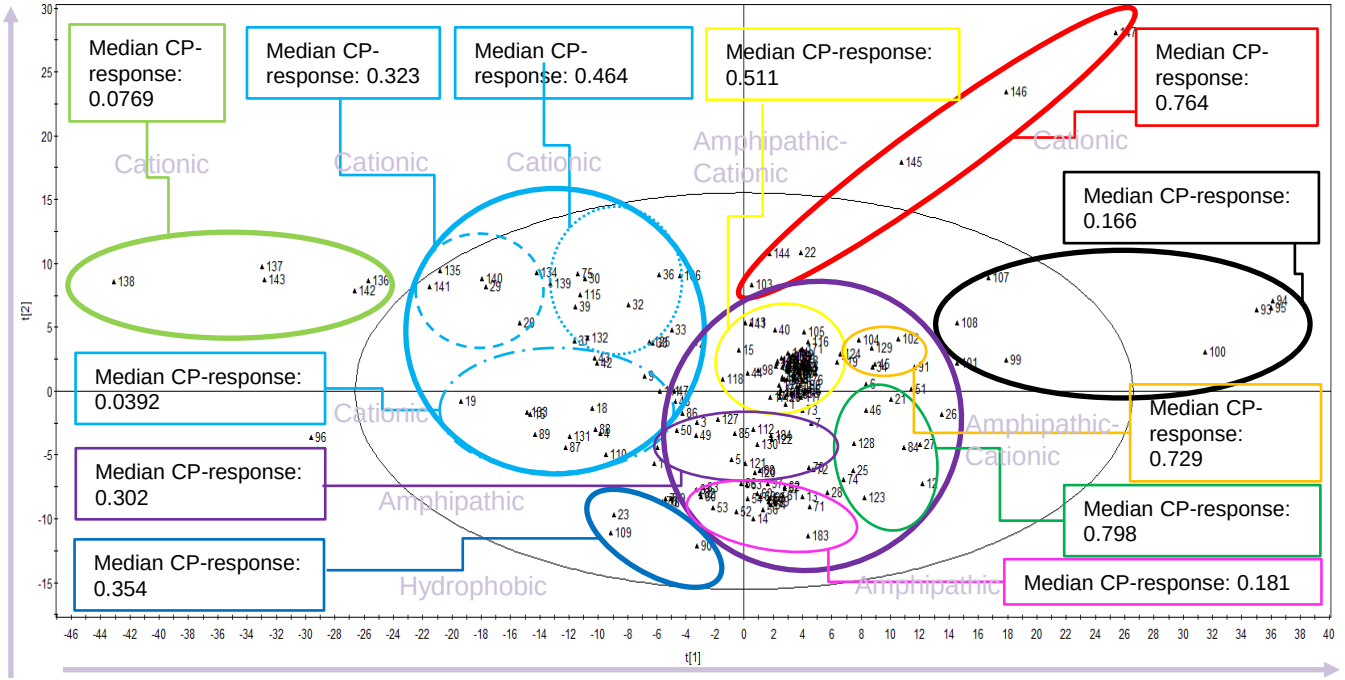
- High MW
- Voluminous
- Complex

DIFFERENT CLUSTERS OF PEPTIDES DIFFER IN CELL-PENETRATING PROPERTIES

Chemical

Functional

- Hydrophilic
- High charge density



Arg-rich

Lys-rich

- Hydrophobic

- Low MW
- Symmetric
- Compact

- High MW
- Voluminous
- Complex

CHEMO-MOLECULAR DESCRIPTORS MOST ROBUSTLY INFLUENCING THE CP-RESPONSE WERE IDENTIFIED USING MLR

- Stepwise MLR model (n = 10)
- *In silico* noised CP-response: 20% variability introduced.

→ Identification of decriptors robustly influencing the CP-response

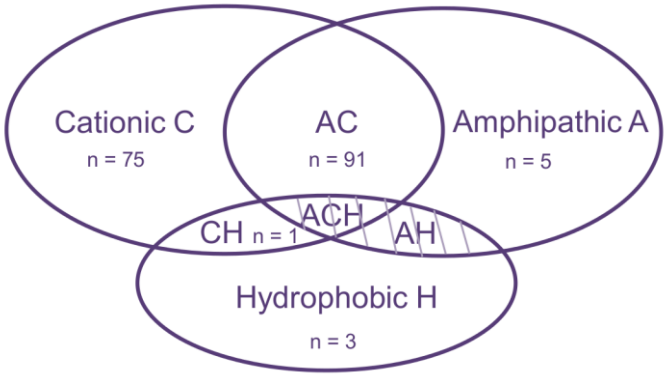
Descriptor	I
G2e	-
G3u	-
nCt	+
nROR	+
Mp	+
T(N..S)	+
B04[N-N]	+
GATS5m	+
GATS7e	+
GATS7p	-
Mor15p	-
Mor26m	-
Mor16p	-
Mor27m	-
Mor27e	+

Descriptor	Meaning	Influence
G2e	2st component symmetry directional WHIM index/weighted by atomic Sanderson electronegativities	-
G3u	3st component symmetry directional WHIM index/unweighted	-
nCt	Number of total tertiary C(sp^3)	+
nROR	Number of ethers (aliphatic)	+
Mp	Mean atomic polarizability (scaled on Carbon atom)	+
T(N..S)	Sum of topological distances between N..S	+
B04[N-N]	Presence/absence of N-N at topological distance 4	+
GATS5m	Geary autocorrelation - lag 5/weighted by atomic masses	+
GATS7e	Geary autocorrelation - lag 7/weighted by atomic Sanderson electronegativities	+
GATS7p	Geary autocorrelation - lag 7/weighted by atomic polarizabilities	-
Mor15p	3D-MoRSE - signal 15/weighted by atomic polarizabilities	-
Mor26m	3D-MoRSE - signal 26/weighted by atomic masses	-
Mor16p	3D-MoRSE - signal 16/weighted by atomic polarizabilities	-
Mor27m	3D-MoRSE - signal 27/weighted by atomic masses	-
Mor27e	3D-MoRSE - signal 27/weighted by atomic Sanderson electronegativities	+

ace

THE CP-RESPONSE IS AN OBJECTIVE AND QUANTITATIVE MEASURE TO RANK CPPs

- Classification of CPPs based on:
  - Origin (*cfr*. M. Lindgren & Ü. Langel, CPPs – Methods and protocols, Humana Press, NY, 2011, 3-19)
  - Physical-chemical properties → clear overlap



- Ranking based on CP-response: objective and quantitative

→ Help discussions

FITTING THE ANTIMICROBIAL PEPTIDES IN THE CHEMICAL SPACE OF CPPs HELPS TO FIND STRUCTURAL AND FUNCTIONAL CORRELATIONS

- Antimicrobial (AMPs) and cell-penetrating peptides (CPPs) investigated as separate group

  ↔ structurally and functionally similar

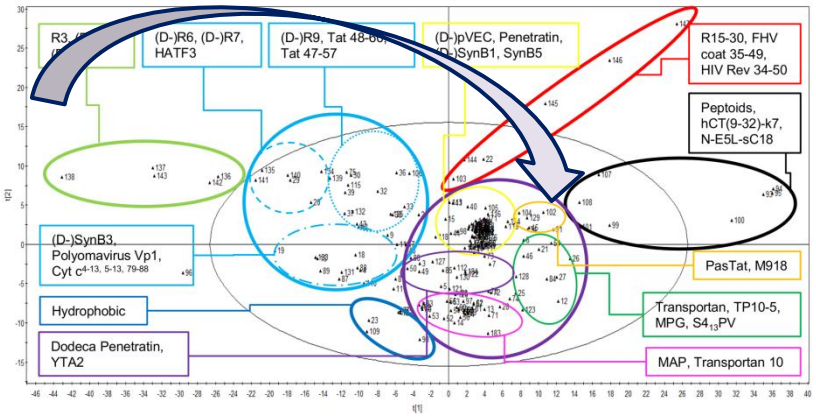
- Fitting AMPs in the chemical space of the CPPs

  → Find structural correlations

  → Unravell uptake mechanism

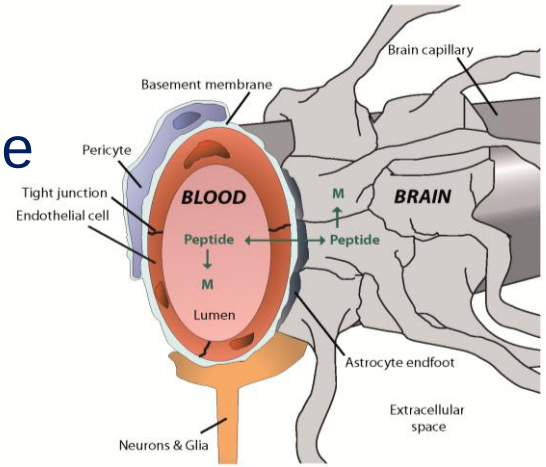
  → *E.g.* short, proline-rich AMPs

  (Protein Peptide Lett, 21(4) ,399-406)



CP-RESPONSE ALLOWS TO FIND A CORRELATION BETWEEN THE CELL-PENETRATING PROPERTIES OF PEPTIDES AND THEIR FUNCTIONALITIES

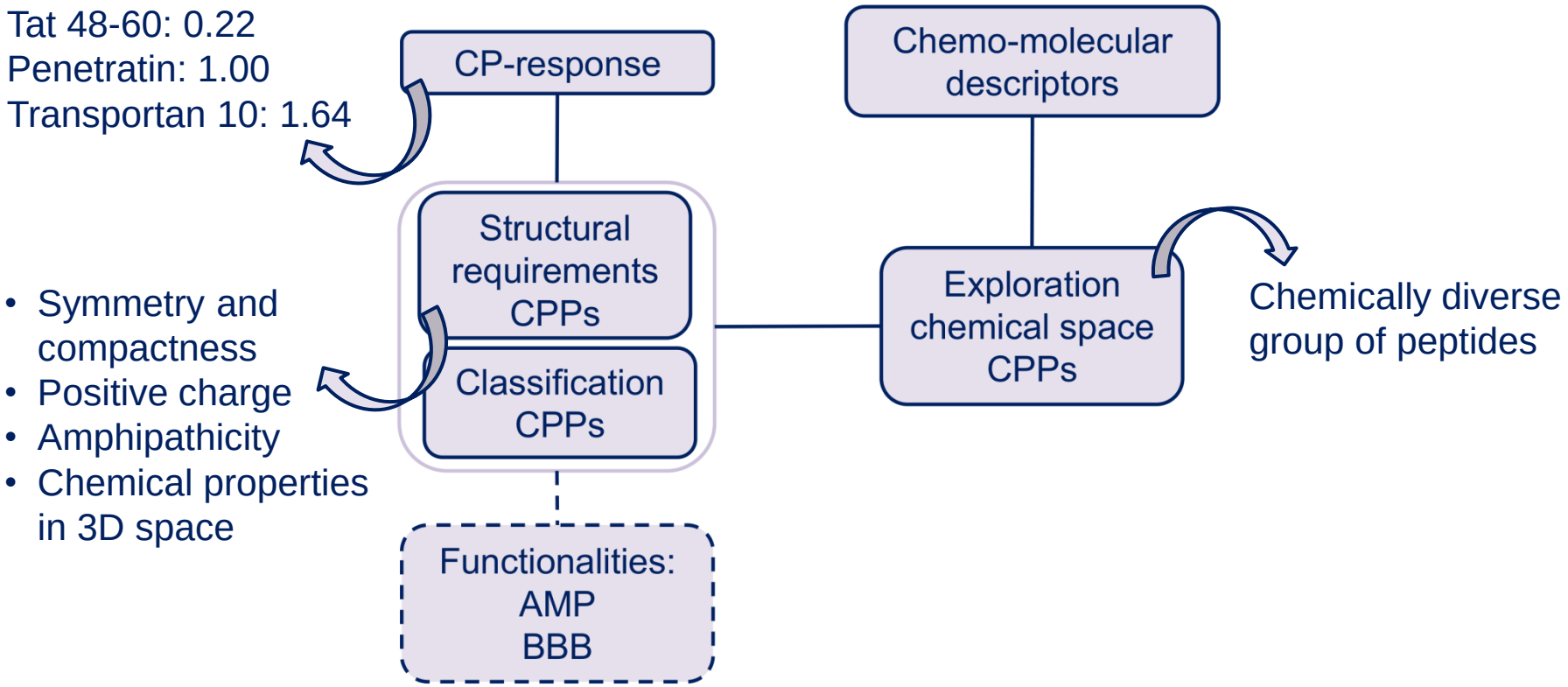
- Blood-brain barrier: selective permeability.
  → Carriers for translocation BBB-impermeable drugs → CPPs?
- *In vivo* blood-to-brain and brain-to-blood transport characteristics of CPPs.



→ 5 CPPs < different clusters chemical space CPPs.

⇨ Cell-penetrating properties of peptides are not a guarantee for BBB influx.

THE EXPLORATION OF THE CHEMICAL SPACE HELPS TO UNDERSTAND THEIR FUNCTIONALITIES



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