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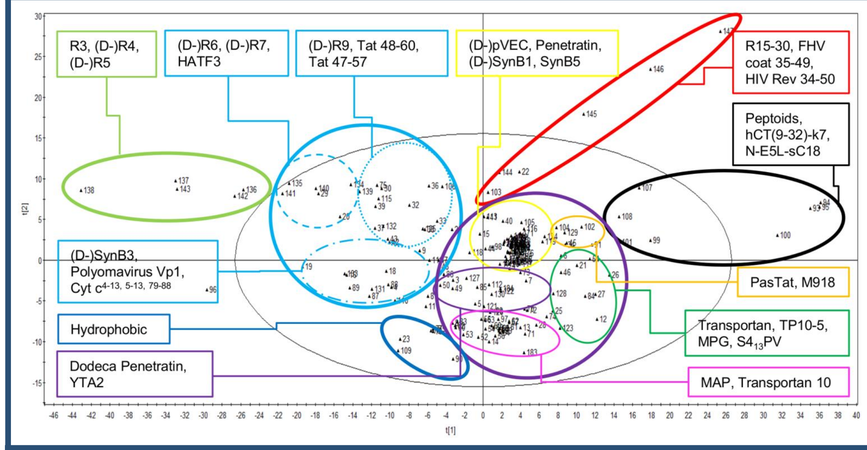
# DO CELL-PENETRATING PEPTIDES CROSS THE BLOOD-BRAIN BARRIER?

**Sofie Stalmans, Evelien Wynendaele, Nathalie Bracke and Bart De Spiegeleer***

**Drug Quality and Registration (DruQuaR) group, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium.**

*Corresponding author: bart.despiegeleer@ugent.be (*O. Ref.*: 2014-358c)

## INTRODUCTION




- Cell-penetrating peptides (CPPs) are a chemically diverse group of peptides and show diverse cell-penetrating (CP) responses, which express their cell-penetrating ability [1].
- In literature, a limited number of studies are available describing CPP-mediated improved transport of different therapeutic molecules across the blood-brain barrier (BBB) *in vivo*.
- Quantitative BBB transport data of uncoupled CPPs are currently lacking.

⇨ **Do all CPPs cross the BBB?**

## EXPERIMENTAL

1. **Selection of model CPPs**
2. ***In vitro* metabolic stability of CPPs** in mouse serum and mouse liver, kidney and brain homogenates
3. ***In vivo* blood-brain barrier transport study** using ICR CD-1 mice:
   - **a. Blood-to-brain transport:** Multiple time regression (MTR) study after IV injection
   - **b. Capillary and parenchymal distribution:** Capillary depletion study after IV injection
   - **c. Brain-to-blood transport:** Efflux study after intracerebroventricular injection

## RESULTS and DISCUSSION

### 1. SELECTION OF MODEL CPPs

Based on:
- Chemically different groups of CPPs in chemical space (see figure introduction)
- CP-response

| Selected peptide | | CP-response | | Chemical class (cluster) |
|---|---|---|---|---|
| Arginine-rich | Tat 47-57 | Low CPP | 0.31 | Cationic (light blue) |
| | SynB3 | | 0.13 | Cationic (light blue) |
| | pVEC | High CPP | 1.32 | Amphipathic-cationic (yellow) |
| Lysine-rich | Transportan 10 | | 1.64 | Amphipathic-cationic (pink) |
| | TP10-2 | | 0.75 | Amphipathic-cationic (pink) |

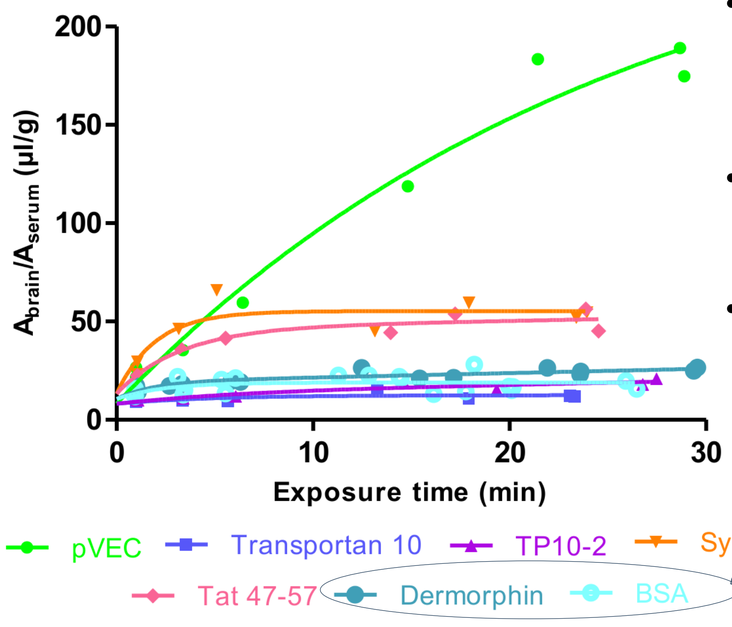
### 2. *IN VITRO* METABOLIC STABILITY OF CPPs

| Peptide | Half life (min) | | | |
|---|---|---|---|---|
| | Serum | Liver | Kidneys | Brain |
| Tat 47-57 | 3 | 60 | 18 | 54 |
| SynB3 | 6 | 37 | 5 | 21 |
| pVEC | < 3 | 43 | 7 | 68 |
| Transportan 10 | 1316 | 139 | 34 | 176 |
| TP10-2 | 229 | 118 | 11 | 102 |

Lower serum stability of arginine-rich CPPs

### 3. *IN VIVO* BLOOD-BRAIN BARRIER TRANSPORT STUDY

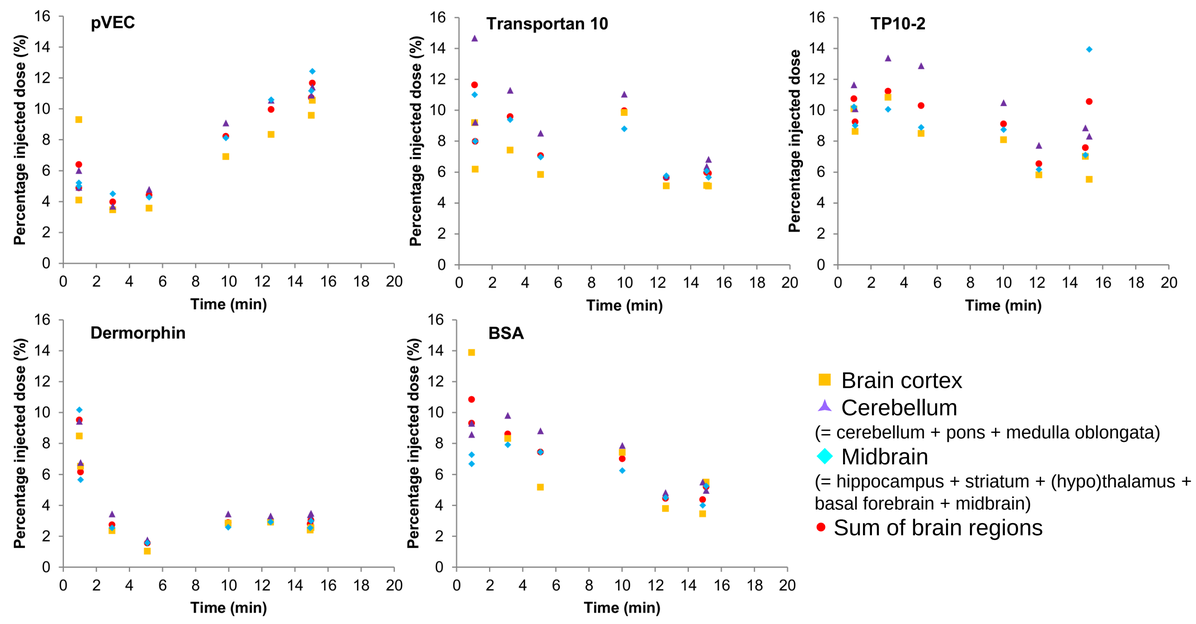
#### a. Blood-to-brain transport (MTR)



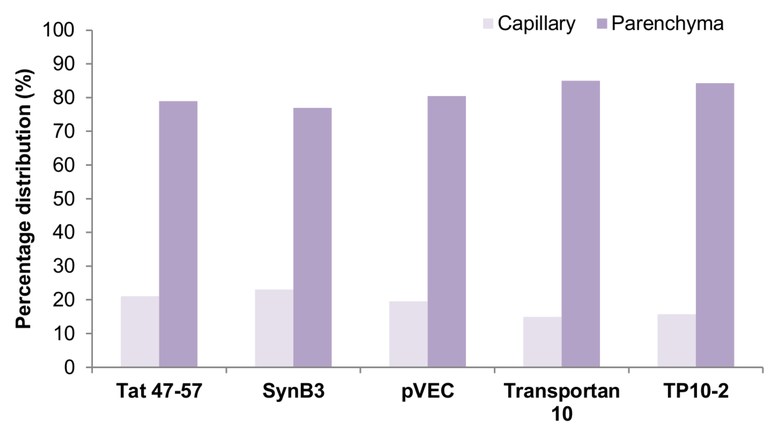

- pVEC shows extraordinary high influx into brain.
- SynB3 and Tat 47-57 show a high brain influx.
- Transportan 10 and TP10-2 have a low to very low brain influx.

Resp. positive and negative control.

- A homogenous distribution to the three main brain regions was observed for pVEC, transportan 10 and TP10-2.




#### b. Capillary and parenchymal distribution




± 80% of the peptide amount that crossed the BBB entered the brain parenchyma.

#### c. Brain-to-blood transport (efflux)

| Peptide | $k_{out}$ ($min^{-1}$) |
|---|---|
| Tat 47-57 | 0.21 ± 0.08 |
| SynB3 | 0.05 ± 0.01 |
| pVEC | 0.10 ± 0.11 |
| Transportan 10 | 0.09 ± 0.02 |
| TP10-2 | 0.06 ± 0.01 |

pVEC: no statistically significant efflux

## CONCLUSION

Good cell-penetrating properties of peptides are not a guarantee for blood-brain barrier penetrating ability.

## REFERENCES

[1] Stalmans S., Wynendaele E., Bracke N., Gevaert B., D'Hondt M., Peremans K., Burvenich C., De Spiegeleer B. (2013) Chemical-Functional Diversity in Cell-Penetrating Peptides. *PLOS ONE*, **8**, e71752.