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Faculty of Chemistry - Organic and Bioorganic Chemistry - OCIII

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Synthesis of cell-penetrating peptides with peroxisomal targeting signal 1

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Peroxisomes

- Peroxisomes play a unique role in metabolism and signalling pathways in all tissues.[1]
- Peroxisomes are involved in many metabolic pathways and thus influence numerous bodily functions.[2]
- Serious diseases such as Zellweger spectrum disorders examplify the importance of peroxisomes and thus the urgency of research in this area.[2]
- The delivery of various non-cellular molecules into the peroxisome is an important tool in peroxisome research.[2]

*adapted from R.J.A. Wanders et al.[2]

CPPs

- Cell penetrating peptides (CPPs) can effectively cross the cell membrane and act as a transporter for cargo molecules up to 200 nm in size.[3]
- CPPs are often positively charged peptides consisting of 2-40 amino acids.[4]
- The different transport mechanisms of CPPs into the cell are divided into two categories: the passive/ direct pathway and the energy-dependent/ endocytotic pathway.[4]

PTS1

- Peroxisomal targeting signal 1 (PTS1) is a C-terminal targeting sequence of peroxisomal matrix proteins.[5]
- PTS1 is recognized in the cytosol by the peroxisomal transport machinery and inserted posttranslationally into the peroxisome.[6]
- Earlier research suggested that PTS1 is a simple tripeptide, with -SKL-OH being a common Sequence. Recent research has shown the influence of the following amino acids and it is now assumed that the effective PTS1 sequence consists of 10-12 amino acids.[7]

Design of a potential transporter

- A CPP and PTS1 were combined to develop a potential in vitro carrier with the goal of being able to penetrate the cell membrane and enter the peroxisomes.

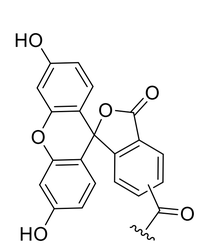
Fluorophore-(G)G-CPP-GG-PTS1-OH

cTAT	Fluorophore-G-G-K-r-R-R-G-r-K-k-R-r-E-G-G-P-V-I-G-S-L-K-S-K-L-OH	1
cR8	Fluorophore-G-G-K-r-R-r-R-r-R-r-R-E-G-G-P-V-I-G-S-L-K-S-K-L-OH	2
KW	Fluorophore-G-K-R-K-R-W-H-W-G-G-P-V-I-G-S-L-K-S-K-L-OH	3
Asparagutin	Fluorophore-G-R-R-W-F-R-R-R-R-R-R-G-G-P-V-I-G-S-L-K-S-K-L-OH	4

Synthesis

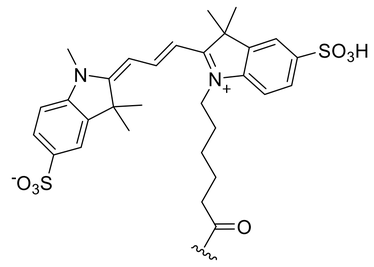
- Through manual SPPS difficulties in the synthesis were optimized
- The final oligopeptides were synthesized via SPPS on a synthesizer
- Cyclisation of the CPP sequence is assumed to enhance the cell penetrating properties and the stability in the cell.[3] A side chain to side chain cyclisation between lysine and glutamic acid was performed for 2 peptides.
- All peptides were labelled with 5(6)-FAM and peptide 3 was also labelled with Sulfo-Cy3 to visualise the subcellular localization of test molecules in human cells.

5(6)-FAM



λ_{ex} = 498 nm
λ_{ex} = 517 nm

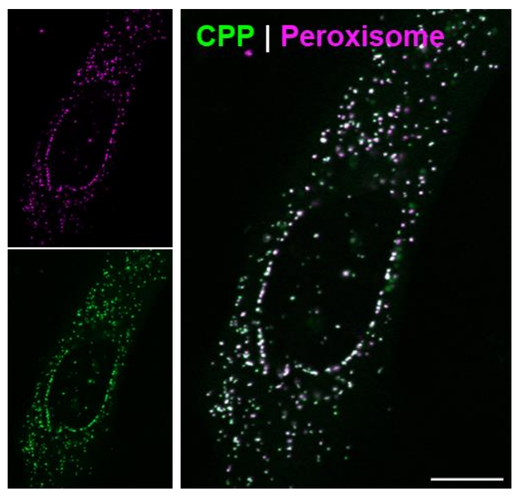
Sulfo-Cy3



λ_{ex} = 548 nm
λ_{ex} = 563 nm

Cell tests

1 h Incubation, 5 µM CPP concentration



U-2 OS, scale bar 10 µm

- Cell tests were performed and analysed with fluorescence microscopy
- All peptides showed low efficiency of peroxisomal localization < 1% (5 µM)
- The peptides entered the cells, but were probably transported endosomally and could not escape from the endosomes
- CPP concentrations of 10 µM showed to be cytotoxic
- CPP conentrations of 0.1 µM showed low signals in the cells

Conclusion and outlook

- The goal was the development of peroxisomal transporters which can penetrate the plasma membrane and are recognized inside the cell to initiate import into the peroxisome. A peptide library of 4 peptides was created with a PTS1 and a CPP part.
- The synthesized transporter 1-4 were tested with cells and analysed with fluorescence microscopy. The analysis indicated a problem with endosomal entrapment of all peptides
- The cell penetrating properties need enhancement to optimize the transporter:
  - The cell penetrating properties of a CPP are influenced by the cargo, the sequence and the concentration of the CPP.[3]
  - Determination of the influence of the PTS1 on the cell penetrating properties on the peptide.
  - Analysis of the structure of the transporters could show if the CPP part is blocked
  - Optimization tests in regards of transporter concentration could be performed.
  - Optimization of the CPP sequence.

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