

## **Novel Cysteine-rich Cell Penetrating Peptide: Efficient Uptake and Cytosolic Localization**

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**Introduction:** Crossing the plasma membrane is a prerequisite for intracellular targeted drug delivery. Cell penetrating peptides are actively used as the delivery tool for intracellular delivery of various cargos. However, confinement of biomolecules into endosomes limits their use for intracellular targeting. Therefore, there is a need for vectors capable of transferring cargo molecules directly into the cytoplasm. Herein, we focus on the development of a novel CPP (derived from polypeptide Crotamine [1]) which shows an efficient uptake at low concentrations ( $\leq 2.5 \mu\text{M}$ ) and cytosolic distribution along with vesicular uptake.

**Methods:** Series of peptides were synthesized by Fmoc strategy, introducing mutations in Cro (27-39) (proposed CPP sequence in Crotamine). All were N-terminally labeled with fluorescein isothiocyanate for optical imaging. Structure Activity Relationship (SAR) studies were done by substitution and/or deletion of amino acid residues in the sequence observing the uptake behaviour by fluorescence spectroscopy and microscopy.

**Results:** Amongst 60 synthesized peptides, one of shorter length showed the best intracellular delivery and cytosolic distribution at lower concentration ( $2.5 \mu\text{M}$ ) when compared to other CPP. Replacing or deleting cysteines had negative impact on internalization. Results also displayed the involvement of tryptophans in cellular uptake indicating, along with cationic amino acids, the importance of each residue in this optimized sequence.

**Conclusions:** SAR studies identified a novel cell penetrating peptide showing, besides of endosomal uptake, also an efficient delivery into the cytoplasm at low concentrations. Thus, this peptide might prove useful for efficient transmembrane delivery of agents directed to cytosolic targets.

[1] Kerkis A. *et al*, *FASEB J.* 2004, 18, 1407.