

## A Novel Cysteine Rich Cell Penetrating Peptide: Gateway to Cytosol

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**Introduction:** Crossing the plasma membrane is a prerequisite for intracellular targeted drug delivery. Cell Penetrating Peptides (CPPs) are potential tools for the intracellular delivery of a wide range of cargos. Though the exact translocation mechanism is still unknown, endocytosis is the most prevalent one seen for highly cationic peptides. 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 Crotonamine [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<sub>(27-39)</sub> (proposed CPP sequence in Crotonamine). All were labeled with fluorescein isothiocyanate at the N-terminal. Structure Activity Relationship studies were done by substitution and/or deletion as well as addition of amino acid residues in the sequence. Uptake of fluorescently labeled peptides were assessed in NIH-3T3 mouse fibroblasts. Effects of varying the labeling concentration, time course, incubation temperature etc. on the intracellular distribution were observed by fluorescence spectroscopy and microscopy.

**Results:** One amongst the various synthesized peptides was identified, showing the best intracellular delivery and cytosolic distribution. Replacing or deleting cysteines and tryptophans had negative impact on internalization indicating along with cationic amino acids the importance of each residue in this optimized sequence. Our novel peptide exhibited mainly cytosolic localization along with some vesicular uptake in cells at a concentration as low as  $2.5 \mu\text{M}$ . Diffused appearance in the cytosol was visible earliest after 4h. A reduction in vesicular uptake was observed on incubation at  $4^\circ\text{C}$  however, cytosolic uptake was almost unaffected, indicating the presence of an additional non-endosomal pathway.

**Conclusions:** Our studies identified a novel peptide that was besides of endosomal uptake, also efficiently delivered into the cytoplasm of the cells. Therefore, it has the potential to be used as CPP for efficient cytosolic delivery of intracellular targeting probes.

[1] Kerkis A. *et al FASEB J.* 2004, 18, 1407-1409.