

**P005** Effects of temperature on intracellular distribution of cell-penetrating peptides

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Cell-penetrating peptides (CPP) are promising candidate vectors for intracellular delivery of therapeutic entities. However, the mechanism of their cellular uptake is still a subject of debate as evidence is proposed for endocytosis and direct translocation across the plasma membrane.

Here we show that the intracellular distribution of two well-studied CPP, octaarginine (R8) and HIV-TAT peptide in hematopoietic cells is dependent on the incubation temperature. At <19°C, fluorescent conjugates of both peptides are diffusely distributed throughout the whole cell, while at 37°C the peptides are primarily localised in endocytic vesicles. Between these temperatures, the cells localise the peptides to both cytosol and endocytic vesicles suggesting they internalise the peptides via two distinct mechanisms. Vesicular labelling characteristic for incubation at 37°C was changed to diffuse labelling in cells incubated with the cholesterol sequestering agent methyl- $\beta$ -cyclodextrin.

Examination of the effects of the peptide chirality on cellular localisation revealed that D-R8 was vesicular at 37°C but at 4°C this variant strongly labelled the nucleolus.

The implications of these findings to our current models of the interaction of CPP with cells and their use as drug delivery vectors will be discussed.