

**P012** An HIV-1 Tat peptide enters Hela cell through the clathrin-mediated endocytosis, not direct plasma membrane penetration

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The cellular entry mechanism of any cell penetrating peptide (CPP) remains unclear and controversial, making rational cargo delivery difficult to design. We studied the cell entry mechanism of R.I.-CKTat9, a proteolytically stable Tat CPP. Fluorescently labeled R.I.-CKTat9 entered Hela cells in an energy- and concentration-dependent manner and assumed both diffused and punctate (vesicular) appearance. Labeled transferrin colocalized with the labeled R.I.-CKTat9 in the punctate structure, suggesting that the peptide enters the cell by the clathrin-dependent endocytosis. Incubation of cells with an isotonic/high K<sup>+</sup> buffer (KPBS) or an NH<sub>4</sub>Cl solution abolished the diffused but not the punctate fluorescence, suggesting that the flux originates from the endosome, not the extracellular space, and relies on the acidity of the endosome. RNA interference of the clathrin heavy chain function and treatments that block endocytosis abolished or greatly reduced both the diffused and the punctate fluorescence, further supporting the sole route of endocytosis and subsequent endosomal escape. Binding of labeled R.I.-CKTat9 to Hela cell surface at 0°C started to fall at the mild acidity of the early endosomes, suggesting an acidity-dependent endosomal escape mechanism and hence our model. These results also suggest that the role of the membrane potential in translocation across either the plasma or the endosomal membranes is minimal.