Models of Cell-Penetrating Peptides

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Short Abstract **— Certain short polycations, such as TAT and polyarginine, rapidly pass through cell membranes and pervade all intracellular compartments by an unknown mechanism called transduction. These cell-penetrating peptides (CPPs) when fused to biologically active peptides promise to be medically useful. A simple model of CPP transduction based on surface tension and the electrostatic field across the membrane of the cell is consistent with the empirical upper limit on the cargo peptide of about 35 amino acids and with experimental data on how the transduction of a polyarginine-fluorophore into mouse C_2C_{12} myoblasts depends on the number of arginines in the CPP and on the concentration of the CPP. The presence of phosphatidylserine on the inner leaflet facilitates the transduction of CPPs. I explain why polyarginines transduce peptides better than do polylysines and compare the inverted-micelle model with experiment.**

In 1988, two groups [1, 2] working on HIV reported that the *trans*-activating transcriptional activator (TAT) of HIV-1 can cross cell membranes. The engine driving this 86-aa cell-penetrating peptide (CPP) is its residues 48–57 which carry a charge of $+8e$. Other CPPS were soon found. Antp is residues 43–58 of Antennapedia, a homeodomain of the fly; it carries a charge of $+7e$. R^n carries charge $+ne$. These and other polycations can penetrate the plasma membranes of live cells towing cargos that greatly exceed the 600 Da restriction barrier.

Many early experiments on CPPs were wrong because the cells were fixed or insufficiently washed or because the fluorescence varied with the (sub)cellular conditions and the fluorophores. Yet some clarity is emerging: TAT carries cargos across cell membranes with high efficiency by at least two functionally distinct mechanisms according to whether the cargo is big or small [3]. Big cargos, such as proteins or quantum dots, enter via caveolae endocytosis and macropinocytosis [4, 5], and relatively few escape the cytoplasmic vesicles in which they then are trapped [3]. Small cargos, such as peptides of fewer than 30–40 amino acids, enter both slowly by endocytosis and rapidly by an unknown mechanism, called transduction, that uses the membrane potential [3, 6–8]. Peptides fused to TAT enter cells within seconds [9] and pervade all intracellular compartments [3].

I first review some therapeutic applications of CPPs and the basics of plasma membranes and then construct a kinetic model of transduction involving surface tension and electrostatics. The model is consistent with the upper limit on the cargo of about 35 amino acids and with the measurements of Tünnemann *et al.* [10] on mouse myoblasts transduced by polyarginines. I explain why polyarginines transduce peptides better than do polylysines and compare the inverted-micelle model with experiment [3].

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