

# CELL-PENETRATING PEPTIDES, ELECTROPORATION, AND DRUG DELIVERY

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## Abstract

Certain short polycations, such as TAT and oligoarginine, rapidly pass through the plasma membranes of mammalian cells by a mechanism called transduction, as well as by endocytosis and macropinocytosis. These cell-penetrating peptides (CPPs) can carry with them cargos of 30 amino acids, more than the nominal limit of 500 Da and enough to be therapeutic. An analysis of the electrostatics of a charge outside the cell membrane and some recent experiments suggest that transduction may proceed by molecular electroporation. Ways to target diseased cells, rather than all cells, are discussed.

## Introduction

We could cure cancer if we knew how to deliver a drug intact to the cytosol of every cancer cell, sparing healthy cells. The circulatory system can deliver a drug to every cell in the body, and certain chemical tricks protect drugs from peptidases and nucleases. But it's harder to cope with antibodies, spare healthy tissues, and get drugs past the plasma membrane, which blocks or endocytoses molecules in excess of 500 Da [1]. This work is about cell-penetrating peptides and other cations that can overcome the 500-Da restriction barrier and about tricks that may spare healthy cells.

## Cell-Penetrating Peptides

In 1988, two groups [2, 3] 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 500 Da restriction barrier.

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 [4]. Big cargos, such as proteins or quantum dots, enter via caveolae endocytosis and macropinocytosis [5], but relatively few escape the cytoplasmic vesicles in which they then are trapped [4]. Small cargos, such as peptides of 30 amino acids, enter both slowly by endocytosis and rapidly by an unknown mechanism, called transduction, that uses the membrane potential [4, 6]. Peptides fused to TAT enter cells within seconds [7].

I review therapeutic applications of CPPs and basic facts about plasma membranes and then describe a model [8, 9] of oligoarginine transduction as molecu-

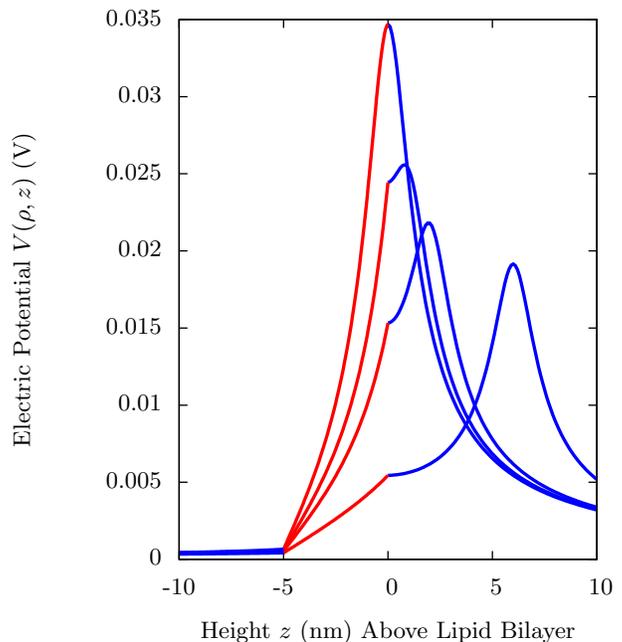


FIG. 1: The electric potential  $V(\rho, z)$  from (1–4) in Volts for  $\rho = 1$  nm as a function of the height  $z$  (nm) above the phospholipid bilayer for a unit charge  $q = |e|$  at  $(\rho, z) = (0, 0)$  (top curve),  $(0, 1)$  (second curve),  $(0, 2)$  (third curve), and  $(0, 6)$  nm (bottom curve). The lipid bilayer extends from  $z = 0$  to  $z = -5$  nm, and the cytosol lies below  $z = -5$  nm. The relative permittivities were taken to be  $\epsilon_w = \epsilon_c = 80$  and  $\epsilon_\ell = 2$ .

lar electroporation. The model is supported by analytic work on the electrostatics of the bilayer, by Monte Carlo simulations of counterions, and by experiments [10]. I sketch a broader class of cell-penetrating molecules and suggest ways to target cancer cells. healthy cells.

## The Potential of an External Charge

The model is based in part upon my analytic calculation of the electric field of a charge just outside the plasma membrane of a eukaryotic cell. The electrostatic potential in the lipid bilayer  $V_\ell(\rho, z)$  due to a charge  $q$  at the point  $(0, 0, h)$  on the  $z$ -axis a height  $h$  above the interface between the lipid bilayer and the extra-cellular

environment is

$$V_\ell(\rho, z) = \frac{q}{4\pi\epsilon_0\epsilon_{w\ell}} \sum_{n=0}^{\infty} (pp')^n \left( \frac{1}{\sqrt{\rho^2 + (z - 2nt - h)^2}} - \frac{p'}{\sqrt{\rho^2 + (z + 2(n+1)t + h)^2}} \right) \quad (1)$$

in which  $t$  is the thickness of the lipid bilayer,  $\epsilon_{w\ell} = (\epsilon_w + \epsilon_\ell)/2$  is average of relative permittivity of the extracellular fluid  $\epsilon_w$  and that of the lipid bilayer  $\epsilon_\ell$ , and  $p$  and  $p'$  are the ratios

$$p = \frac{\epsilon_w - \epsilon_\ell}{\epsilon_w + \epsilon_\ell} \quad \text{and} \quad p' = \frac{\epsilon_c - \epsilon_\ell}{\epsilon_c + \epsilon_\ell} \quad (2)$$

which lie between 0 and 1,  $\epsilon_c$  being the relative permittivity of the cytosol. The potential in the extra-cellular medium is

$$V_w(\rho, z) = \frac{q}{4\pi\epsilon_0\epsilon_w} \left( \frac{1}{r} + \frac{p}{\sqrt{\rho^2 + (z + h)^2}} - \frac{\epsilon_w\epsilon_\ell}{\epsilon_{w\ell}^2} \sum_{n=1}^{\infty} \frac{p^{n-1}p'^n}{\sqrt{\rho^2 + (z + 2nt + h)^2}} \right) \quad (3)$$

in which  $r = \sqrt{\rho^2 + (z - h)^2}$  is the distance from the charge  $q$ . The potential in the cytosol due to the same charge  $q$  is

$$V_c(\rho, z) = \frac{q\epsilon_\ell}{4\pi\epsilon_0\epsilon_{w\ell}\epsilon_{\ell c}} \sum_{n=0}^{\infty} \frac{(pp')^n}{\sqrt{\rho^2 + (z - 2nt - h)^2}}. \quad (4)$$

where  $\epsilon_{\ell c}$  is the mean relative permittivity  $\epsilon_{\ell c} = (\epsilon_\ell + \epsilon_c)/2$ .

## Monte Carlo Simulation of the Counterions

The model also is based upon my use of Monte Carlo methods to compute the transmembrane potential  $\Delta V_{NaCl}$  due to the sodium and chloride ions of the extracellular medium near an oligoarginine.

The  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{++}$ ,  $\text{Ca}^{++}$ , and  $\text{Cl}^-$  concentrations in the extracellular medium respectively are 145, 5, 1–2, 1–2, and 110 mM. I approximated their effects by setting the  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations to 156 mM and ignoring the other ions. I used an active volume that was 10 nm wide and 20 nm long, and that rose from the lipid bilayer to a height of 5 nm. In this active volume of  $200 \text{ (nm)}^3$ , I put 94 sodium ions and  $(94+n)$  chloride ions so as to make the charge within the active volume neutral.

To prevent the sodium and chloride ions from avoiding the walls and ceiling of the active volume, I surrounded the walls and ceiling of the active volume with a  $1000 \text{ (nm)}^3$  5 nm-thick passive volume in which I randomly placed 470  $\text{Na}^+$  and 470  $\text{Cl}^-$  ions.

The Monte Carlo code used the potential  $V_w$  of Eq. (3) to compute the energy of an individual sodium or chloride ion in the active volume due to its interaction with all the ions in the active and passive volumes and with the CPP(s) which did not move. The fixed positions of the  $n$  charges of the oligoarginine depended upon whether the  $\text{R}^n$  was configured as an  $\alpha$ -helix, a random coil, or a  $\beta$ -strand. The ions in the passive volume also didn't move, retaining their original random positions, which were different in each run.

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