Arginine-rich, cell-penetrating peptides (CPPs) are one of the most potent drug delivery tools to carry therapeutic molecules into cells. However, the internalization mechanism of CPPs across hydrophobic cell membranes is still remains unclear and controversial. In this thesis, the mechanism of membrane penetration of polyarginine was investigated from physicochemical aspects.

The results demonstrated that polyarginine binds to the membrane interface region, with the degree of insertion being greater for the longer polyarginine. Such lipid interactions induce the transition from a random coil to the α-helix structure of the longer polyarginine whereas no structural change was observed for the shorter polyarginine. The contribution of the favorable enthalpy to the energetics of lipid binding of polyarginine increases with the increase in the polymer chain length. In addition, polyarginines penetrate across giant vesicle membranes, and the amount of membrane penetration increases with increasing the polymer chain length. On the basis of these observations, it appears that the enhanced ability of the longer polyarginines to translocate lipid membranes is due to their greater perturbation of the membrane structure. Thus, the formation of α-helical structure upon lipid binding drives the insertion of polyarginine into the membrane interior, which enhances the membrane penetration of polyarginine.

We also performed real time in-cell NMR study to obtain the quantitative physical parameters for the membrane penetration of $^{19}$F-labeled octaarginine in cells in situ. Our methodology quantitates the rate constant, equilibrium constant, change in Gibbs energy, and even the amount of transmitted CPP into cytosol of HL60 cells. Our theoretical calculation for the amount of the translocation into cytosol of $^{19}$F-R8 is also consistent with experimental quantification. These results showed that once the cationic nature of R8 was cancelled by binding to anionic GAG, the transfer to the lipid membrane proceeded most rapidly (7.5 min$^{-1}$). In contrast, the rate constant of the entry into cytosol was 0.31 min$^{-1}$, more than one order of magnitude as slow as the penetration into the membrane. Almost no energy was changed, however, for the entry of cationic R8 into cytosol across the hydrophobic membrane.