Peptide conjugates of PNA as steric block modulators of gene expression

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Peptide nucleic acids (PNA) are charge neutral oligonucleotide analogues that form strong complementary hybrids with RNA targets and that have been used to modulate gene expression inside cells. We have been developing the use of cell penetrating peptides (CPP) as covalent conjugates of PNA to promote cell uptake and improved delivery through enhancement of release from endosomal compartments. We found recently that conjugation of a PNA complementary to the HIV-1 TAR sequence with an arginine-modified Penetratin (R₆-Penetratin) allowed delivery into HeLa cell nuclei and inhibition of Tat-dependent gene expression in a luciferase reporter assay. We report now that similar R₆-Penetratin-PNA conjugates complementary to the 705 splice junction site in a firefly luciferase up-regulation model using HeLa pluc705 cells gave rise to extremely high (50-70%) splicing correction when added at just 1 µM for 4 hours in the absence of any endosomolytic reagent. Such correction is much higher than obtained with PNA conjugates of standard CPPs (e.g. Tat(48-60), Penetratin, Transportan, (Lys)ₙ). We report preliminary structure-function analysis aimed at characterizing the features of the peptide conjugate important to high splice correction activity. We are also using the R₆-Penetratin-PNA paradigm towards the development of new reagents to inhibit the activity of biologically important microRNA targets.