Today, the efficient delivery of nucleic acid as therapeutic agent is a major challenge in gene therapy. Peptides have emerged as a novel carrier for the delivery of drugs and genes. Peptides are used due to properties such as efficiency of cell penetration and capacity to bind and deliver. Cell-Penetrating Peptides (CPPs) are short peptides, with the ability to gain access to the interior of biological cells. They have the exceptional ability of carrying into the cells a wide variety of covalently and non-covalently conjugated cargoes such as proteins, oligonucleotides, and even liposomes. Hence, they are extremely attractive candidates for the transport drugs to the interior of cells. C6M1 amphipathic peptide as a modified version of the C6 peptide for siRNA delivery. The modification of C6 peptide by introducing tryptophan residue change solubility, secondary structure, cytotoxicity and cellular uptake efficiency. C6 and C6M1 CPPs interactions with GADPH siRNA were investigated using computational methods.

**Figure 01: C6M1 cell penetrating peptide**

**Figure 02: Leucine residues in α-helix structure are substituted by tryptophan residues of C6M1 α-helix structure.**

Some of the aliphatic pendent groups in the hydrophobic face of C6 are substituted with aromatic groups to yield the C6M1 peptide. Here three leucine residues in C6, leu3, leu7 and leu14 have been replaced by tryptophan residues in C6M1. When considering these amino acids, arginine residues interact with the negatively charged cell membrane phospholipids and also form non-covalent complexes with negatively charged cargos such as siRNA, via electrostatic interactions. Leucine residues can interact with the hydrophobic tails of the lipid bilayer, facilitating the translocation of the peptide. The aromatic tryptophan residues can enhance the cellular uptake of arginine rich peptides. In the modified version, the arrangement of all tryptophan residues is at the same face of the helix which also stabilizes the helical structure through π-π interactions between tryptophan rings.

Small interfering RNA (siRNA), also known as short interfering RNA or silencing RNA, is a class of double-stranded RNA molecules, 20-25 base pairs in length. siRNA plays many roles, but its most notable role is in the RNA interference (RNAi) pathway, where it interferes with the expression of specific genes with complementary nucleotide sequence. Hence, small interfering RNA (siRNA) is a very powerful tool that can interfere with and silence the expression of specific disease gene. To improve cellular uptake of siRNA, CPP strategies have been applied to facilitate the delivery of siRNA into cells through either covalent or non-covalent linkages. The following siRNA molecule was considered during the research.

- **Sense (guide) sequence**
  5’ GACUUAAAGGCACAGAAUC3’
  21 base pairs

- **Antisense (passenger) sequence**
  5’ AGCGGUGUUGGUCAGG3’
  19 base pairs

where, A,a adenine, C,cycosine, G,guanine, U,uracil

**RESULTS AND DISCUSSION**

- **Hydrophobicity**
  The ideal α-helix structures of C6 and C6M1 were generated. Visualization of these structures (using VMD) showed that the hydrophobic and hydrophilic residues of the peptide aligned in opposite directions in the secondary structure. Tryptophan is considered to be more hydrophobic than leucine amino acid. Furthermore, arginine has the lowest hydrophobic nature and hence more polar properties. Increased hydrophobicity is considered advantageous for CPPs for crossing the hydrophobic cell membranes.

**Figure 05: Helix plot**

- **Secondary structure and helicity**

**Figure 06: (i)/(ii) C6M1 α-helix with si-RNA complex**

- **The binding energies (B.E) of the C6 alpha and C6M1 alpha structures with si-RNA molecule**

<table>
<thead>
<tr>
<th>Compound</th>
<th>B.E (KJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6 alpha</td>
<td>(-2164.8434)</td>
</tr>
<tr>
<td>C6M1 alpha</td>
<td>(-2115.4812)</td>
</tr>
<tr>
<td>siRNA</td>
<td>(-1182.2436)</td>
</tr>
</tbody>
</table>

**CONCLUSION**

- C6M1 CPP is more hydrophobic than C6 CPP.
- The secondary structure of C6M1 CPP was proved to be α-helical and it shows an increment of helicity.
- The binding energies of C6 CPP and C6M1 CPP are almost same with si-RNA. Modification has not caused to increase the binding energy with C6M1 CPP hence no damage to the arginine binding sites of the CPP with si-RNA molecule.

**REFERENCES**