INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	MAHESH AGARWAL
Roll Number	:	126106022
Programme of Study	:	Ph.D.

Thesis Title: **"**Studies on Recombinant Protein and Peptides derived from Diphtheria Toxin**".**

Name of Thesis Supervisor(s)	:	Dr. Biplab Bose
Thesis Submitted to the Department/ Center	:	Dept. of Biosciences and Bioengineering
Date of completion of Thesis Viva-Voce Exam	:	2nd April 2018
Key words for description of Thesis Work	:	Diphtheria toxin, Targeted Delivery, Nanoparticles, Endocytosis, HB-EGF, β-hairpin peptides.

SHORT ABSTRACT

Diphtheria toxin (DT) is a well characterized AB-toxin with three independent domains: C-domain for catalysis and toxicity, T-domain for translocation through the membrane and R-domain or receptor-binding domain. The toxicity of DT has been investigated extensively and is utilized to create therapeutic agents, like immunotoxins, which kill cells. However, the receptor-binding ability of DT is not extensively explored and used for therapeutic purposes.

The receptor of DT is Heparin-binding EGF-like growth factor (HB-EGF). It is expressed as a membrane-bound molecule, which is eventually released by ectodomain shedding. HB-EGF is a growth factor. It is overexpressed in various cancer cells and activates different oncogenic signaling pathways. Therefore, HB-EGF on the cell surface can be targeted for cell-specific drug delivery. It can also be targeted to modulate its oncogenic signaling.

In the current work, we have manipulated the receptor-binding domain of Diphtheria toxin (RDT) in two ways. First, we have used recombinant RDT to deliver drug-loaded nanoparticles to specific cells that express Human HB-EGF. In the second part, we have established that a short stretch of 26 amino acids in RDT is adequate for binding to HB-EGF with moderate affinity.

We have synthesized polymeric PLGA nanoparticles (NPs) and coated those with recombinant RDT. These RDT-coated NPs (RDT-NPs) were characterized by TEM, SEM, DLS, FTIR, and ELISA. Using flow cytometer and spectrofluorimetry-based experiments, we show that RDT-NPs has enhanced uptake in cells expressing human HB-EGF and such uptake involve Clathrin-dependent receptor-mediated endocytosis. We further show that this receptor-targeted delivery through RDT-NPs increases the potency of a chemotherapeutic agent.

Subsequently, we have attempted to create RDT-derived peptides that would bind to HB-EGF. Such peptides can be utilized for HB-EGF-targeted drug delivery or to modulated HB-EGF signaling. We analyzed the structure of RDT and identified a stretch of 26 amino acids that is crucial for binding to HB-EGF. Based on such structural information, we designed three 26 amino acid long peptides. We performed docking and molecular dynamic simulations to understand possible structural features and the receptor-binding ability of these peptides. We synthesized these peptides by solid phase synthesis and characterized those through several techniques like ESI mass spectrometry, and CD spectroscopy. Subsequently, we show that these peptides bind to HB-EGF. Further, we expressed these peptides in *E. coli* as MBP-tagged peptides. All three MBP-tagged peptide retained HB-EGF binding, and SPR-based analysis showed that these MBP-tagged peptides bind to HB-EGF with moderate affinities.