INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: **Establishment of a bioactive recombinant protein toolbox for the prospective generation of integration-free induced pluripotent stem cells and other biological applications**

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SHORT ABSTRACT

The derivation of induced pluripotent stem cells (iPSCs) over a decade ago ushered a new era in the cellular reprogramming paradigm. The very concept of reprogramming somatic cells into a pluripotent cell stage brought great enthusiasm in the scientific community as it opened new and promising avenues in the field of disease modeling, drug designing, understanding the intricacies of developmental biology, and wide use of autologous cell-based therapies. The generation of iPSCs enhances the prospects of pluripotent cells from bench to bedside, providing an opportunity to bring patient-specific therapies. However, the major limitation was the use of integrative approaches for the generation of iPSCs that drastically crippled their use in clinical applications due to random integrations, leading to mutations. Alternative to the integrative approaches, the non-integrative approaches provided a fruitful solution to overcome the problem of genomic integration. Over the years, several non-integrative approaches have emerged to generate integration-free iPSCs; among them, the recombinant protein-based approach is deemed to be the safest one. There are several challenges associated with protein-based cellular reprogramming. Hence, we aim to establish a recombinant protein toolbox (OCT4, SOX2, UTF1 and GLIS1) for the generation of integration-free human iPSCs by addressing the roadblocks. In this study, we have laid down simple and methodical strategies to generate recombinant proteins withholding native-like secondary structure conformations, thereby retaining their functionality. The generated recombinant proteins were delivered into mammalian cells and showed successful cellular and nuclear translocation. We showed that the generated recombinant fusion proteins are biologically active, as OCT4 showed reduction in cell migration and cell proliferation in human fibroblasts. Additionally, we also confirmed the biological activity of the purified OCT4 protein using a reporter system, where this protein binds to its own promoter, thereby expressing GFP. SOX2 and UTF1 showed tumorigenic and tumor-suppressive roles in cervical cancer cells, respectively. GLIS1 showed tumorigenic potential in breast cancer cells, with no significant effect on normal human fibroblasts. The established functionally active recombinant protein toolbox provides a path to generate integration-free iPSCs circumventing genetic manipulation. These proteins also open various opportunities to unravel their functions in different cancers and their potential as promising therapeutic targets and other biological applications in the near future.