INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Programme of Study : **Ph.D.**

Thesis Title:
Establishment of a bioactive recombinant protein toolbox for the prospective generation of integration-free cardiomyocytes and other biological applications

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SHORT ABSTRACT

Direct reprogramming or direct conversion of somatic cells to functional induced cardiomyocytes bypassing the pluripotent state offers a promising alternative in cell therapies. The major problem with the generation of cardiomyocytes using traditional integrative approaches is limiting its application of clinical therapies. Therefore, the main focus now shifted towards finding a clinically relevant non-integrative approach for direct cardiac reprogramming, considering the risk factors associated with integrative approaches. In this prospect, recombinant protein-based cellular reprogramming has been reported as the safest approach among other approaches. Although recombinant protein-based therapeutics or cellular reprogramming is a safer and more promising alternative, there are several challenges associated with the heterologous production of these recombinant proteins. Therefore, we aim here to establish a recombinant protein toolbox consisting of key cardiac reprogramming transcription factors (ETS2, MESP1, GATA4, MEF2C, TBX5, and HAND2) by addressing those associated problems for the prospective generation of integration-free functional cardiomyocytes. The recombinant proteins generated in this research by employing simple and systematic strategies will have native-like folding conformations, thereby retaining their biological activity. In addition, the generated recombinant proteins can be directly delivered to the mammalian cells and can be localized into the nucleus without any requirement of transduction reagents. Interestingly, we found MESP1 to be an ion-sensitive protein. Using a reporter system, we confirmed the synergistic effect of purified MEF2C and HAND2 proteins in the activation of alpha-Myosin Heavy Chain, thus suggesting these factors may contribute to the maturation of cardiomyocytes. Additionally, we also confirmed that the ETS2, MESP1, and GATA4 factors play crucial roles in breast cancers, whereas TBX5 plays a crucial role in colon cancer. In the near future, this established recombinant protein toolbox will pave the way to generate transgene-free patient-specific autologous and functional cardiomyocytes with no involvement of genetic manipulation.