NANYANG TECHNOLOGICAL UNIVERSITY SINGAPORE

School of Biological Sciences
College of Science

Reg. No. 200604393R

Research Theme: Biotechnology

MSc Research Project Title:

Systematic Engineering of Terminal Deoxynucleotidyl Transferase

Principal Investigator/Supervisor: Wu Bin

Co-supervisor/ Collaborator(s) (if any):

Project Description

a) Background:

Terminal Deoxynucleictidyl Transferase (TdT) catalyzes termplate-free single strand DNA synthesis and 3' terminal modification. As compared to widely used DNA phosphoramidite based chemical synthesis method, TdT enzyme offers the following advantages:

1. Theoretically high processivity that produces longer DNA more efficiently.
2. Higher chemical compatibility with a variety of different DNA nucleotide analogues.
3. Efficient 3' end labelling.

In a recent study [1], Prof. Ma described his strategies in optimizing the design of ZaTdT for potential commercial applications. However, from a structural biologist point of view, much of his conclusions were drawn based on computer simulated models. In particular, the critical catalytic loop residues 388-399 was very flexible (and hence its structure was uncertain) in all of the models he used. As shown by his own data, many of the intended gain-of-function mutants has insignificant improvement of catalytic activity as compared to wild type ZaTdT. We propose to conduct structure-based enzyme optimization of TdT to hopefully identify a more efficient variant of this enzyme.

1. Enzymatic DNA Synthesis by Engineering Terminal Deoxynucleotidyl Transferase
Xiaoyun Lu, Jinlong Li, Congyu Li, Qianqian Lou, Kai Peng, Bijun Cai, Ying Liu, Yonghong Yao, Lina Lu, Zhenyang Tian, Hongwu Ma, Wen Wang, Jian Cheng, Xiaoxian Guo, Huifeng Jiang, and Yanhe Ma
ACS Catalysis 2022 12 (5), 2988-2997
DOI: 10.1021/acscatal.1c04879

b) Proposed work:

We propose to test different strategies of structural based mutagenesis and evolution of TdT to obtain these more efficient/specialized isoforms.
Aim1: To optimize the amino acids of TdT, based on an actual atomic structure of TdT.
Aim2: To establish a high-throughput screening platform to identify more enzymatically efficient TdT.

c) Preferred skills:

Biochemistry, molecular biology

Supervisor contact:

If you have questions regarding this project, please email the Principal Investigator:

SBS contact and how to apply:

Associate Chair-Biological Sciences (Graduate Studies) : AC-SBS-GS@ntu.edu.sg

Please apply at the following:

Application portal:

https://venus.wis.ntu.edu.sg/GOAL/OnlineApplicationModule/frmOnlineApplication.ASPX