NANYANG TECHNOLOGICAL UNIVERSITY SINGAPORE

School of Biological Sciences
College of Science

Reg. No. 200604393R

Research Theme: **Synthetic Biology**

MSc Research Project Title: **Using Synthetic Biology to Reconstruct the Origins of the Genetic Code**

Principal Investigator/Supervisor: **Richard She**

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

Project Description

a) Background:

The triplet nature of the genetic code—the mapping of 64 codons to 20 amino acids—is one of the most ancient and conserved features of biology. Every known organism, from bacteria to humans, uses essentially the same codon table, with only rare, minor deviations. Francis Crick famously referred to this as a "frozen accident": once the translational machinery became sufficiently complex, further changes to the code were too deleterious to tolerate, locking in a specific mapping that persists to this day.

Yet the code is not random. Many codons that differ at a single base encode the same amino acid (*synonymous substitutions*), and even when amino acids change (*non-synonymous substitutions*), the replacements tend to be chemically similar—preserving properties like hydrophobicity, charge, or size. This structure likely reflects strong selection for mutational robustness, especially if early translation or replication was error-prone.

Despite decades of speculation, we still lack an empirical framework for understanding how the modern genetic code could have evolved. A 20-amino acid code must have been built incrementally—through a series of viable intermediate codon tables with fewer amino acids and simpler tRNA pools. But which intermediate codes are biophysically plausible? Which subsets of amino acids are sufficient to produce structured, functional proteins? And in what order might the amino acid repertoire have expanded?

Our goal is to experimentally reconstruct and test such intermediate codes using modern tools in synthetic biology.

b) Proposed work:

Incoming Ph.D. students will lead an experimental effort to design and test simplified versions of the genetic code. The goal is to identify reduced amino acid alphabets and codon tables that can still produce structured, functional proteins.

We will build and test "sub-codon tables"—synthetic versions of the genetic code that use only a subset of codons and amino acids. For example, we might restrict the third codon position to just C or G (called an "SNN" library), which dramatically reduces the amino acid diversity in resulting proteins. Using in vitro translation, we will synthesize vast libraries of proteins (~10^{12} variants) constrained to these reduced codes, then perform affinity-based selections to identify proteins that fold or bind RNA.

In parallel, we will deliver these codon-constrained libraries into mammalian cells at scale (~100 million cells per library) using lentiviral vectors. Functional selection will occur through fluorescence-based reporters: for example, fusion to a split-GFP domain, where proper folding allows GFP reconstitution and organelle localization. Cells with correctly folded proteins will be isolated by flow cytometry.

These complementary assays will allow us to explore which subsets of the genetic code can still produce biochemically functional proteins—and whether some code trajectories are more robust or evolvable than others. Students will gain training in synthetic library design, in vitro transcription/translation, lentiviral delivery, pooled screening, flow cytometry, and deep sequencing-based readouts. This project lies at the intersection of molecular evolution, synthetic biology, and protein engineering.

c) Preferred skills:
Students should be excited about fundamental questions in molecular biology and evolution. No specific prior experience is required, but familiarity with molecular cloning or coding (Python/R) will be helpful.

Supervisor contact:

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

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SBS contact and how to apply:

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Please apply at the following:

Application portal:

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