Abstract:

Cellular life as we know it is dependent on all 20 canonical amino acids comprising the majority of all proteins. Study of origins of life has implied that life might have begun its existence with a smaller alphabet. Although imaginable as a hypothetical concept, providing experimental evidence for such organism has proven to be a difficult task, as our understanding of life's many intricacies as well as rational protein design is lacking.

Recent advancements in the combinatorial genome editing and *in silico* protein folding have the potential to open the path towards experimental reductions of the amino acid code. As for a first step in this reduction tryptophan was chosen here as a suitable candidate as this amino acid is currently thought to be among the latest additions to the alphabet. It is one of the least represented amino acids (comprising ~1.5 % of amino acids in *Escherichia coli*), is coded by one codon. Using Multiplex Automated Genome Engineering (MAGE), a biosynthetic pathway of tryptophan in *Escherichia coli* was completely deprived of tryptophan. This was achieved by the introduction of an amino acid library lacking tryptophan in the tryptophan coding sites of this operon. Although the reduction in the amino acid alphabet was not massive, it may serve as an indication for the dispensability of tryptophan in cellular life. The successful removal of tryptophan from a biosynthetic pathway also serves as a stepping stone for further cellular minimisation.

Key words: Amino acid dispensability, Evolution of genetic code, Tryptophan, Recombineering, Genome engineering