

# Review of: "Measuring the tolerance of the genetic code to altered codon size"

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Some ideas for the authors consideration:

Quadruplet tRNAs (qtRNAs) have some potential defects that do not appear to be fully explained in this paper. First of all, the anticodon loop is strongly selected to form a 7-nt loop, not an 8-nt loop. I know there are exceptions. The 7-nt loop forms a U-turn between loop positions 2 and 3. The 7-nt loop forms a reasonably stiff RNA loop that presents a 3-nt anticodon. The 8-nt loop used for qtRNAs in the present study would be overly flexible compared to a tRNA 7-nt loop and might disrupt the U-turn configuration of the tRNA.

There is no analysis of the modifications of the anticodon loop or the tRNA-37 position in this paper. TRNA-37 modifications enable reading of the tRNA-36 position, suppress frame-shifting and delimit the anticodon. With qtRNAs, patterns of tRNA modifications may be relevant to the reading of unnatural qtRNAs. Building an entire code with 256 codons and qtRNAs would present (probably) insurmountable difficulties. TRNA evolved to support a 3-nt anticodon. If a goal of the future is to generate a genetic code using additional amino acids, perhaps another strategy might be to try to split existing 4-codon and 6-codon boxes in the established 3-nt code.

These objections aside, the paper is very interesting. The constructions are rather beautiful and the tests are well-presented. The authors have done some rather elegant engineering and analyses. This reviewer has no criticisms of the methodology or presentation of data.