

## Supplementary Information

### Twice exploration of tRNA +1 frameshifting in an elongation cycle of protein synthesis

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Figure S1

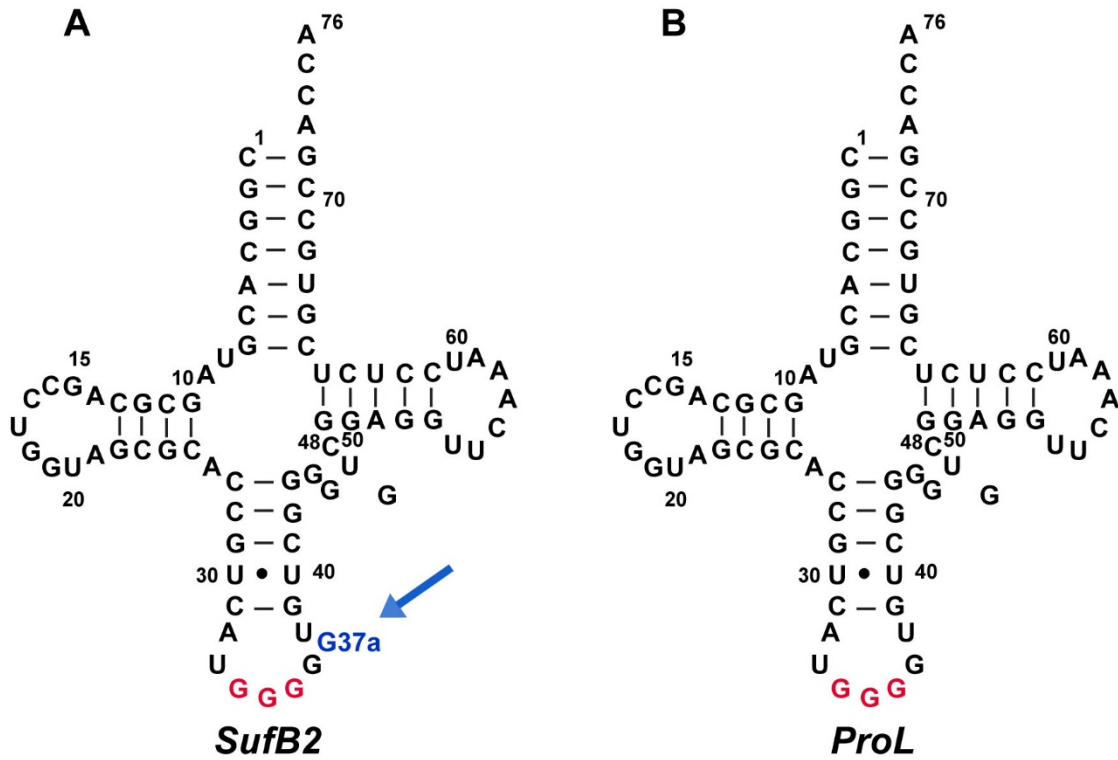


Figure S1. Sequences and cloverleaf structures of *Salmonella SufB2* and *ProL* tRNAs, which are synthesized by *in vitro* transcription for this study. *SufB2* has the insertion of G37a next to G37 in the anticodon loop of *ProL*. The anticodon GGG in both tRNAs are shown in red.

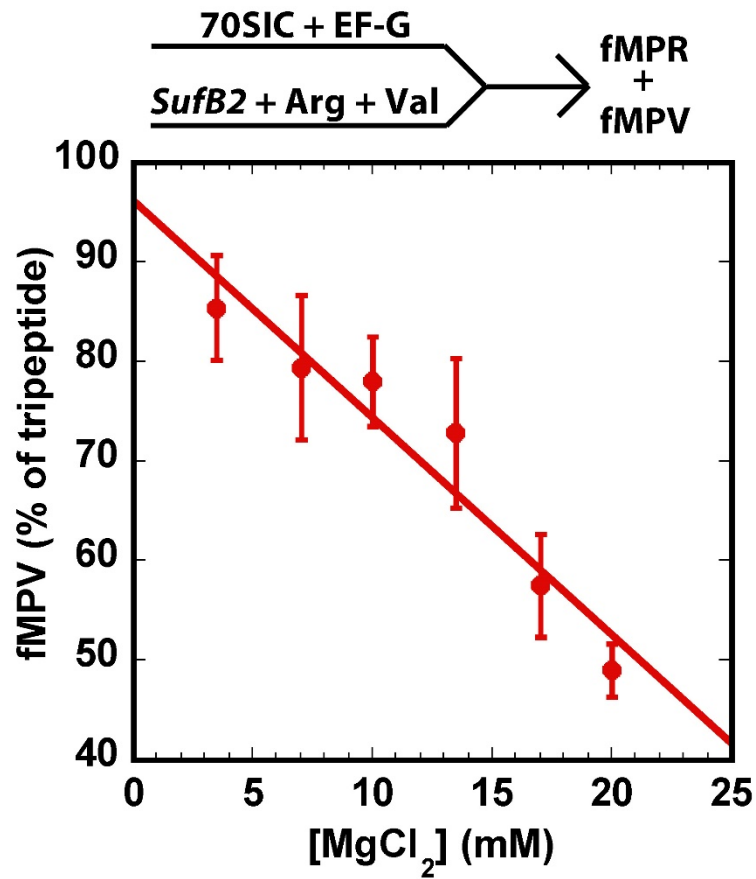


Figure S2. A kinetic assay for the fractional distribution (%) of fMPV after translocation of *SufB2* into the P site as a function of  $Mg^{2+}$  concentration. This fractional distribution represents the sub-population of *SufB2* in the +1-frame of the P site.

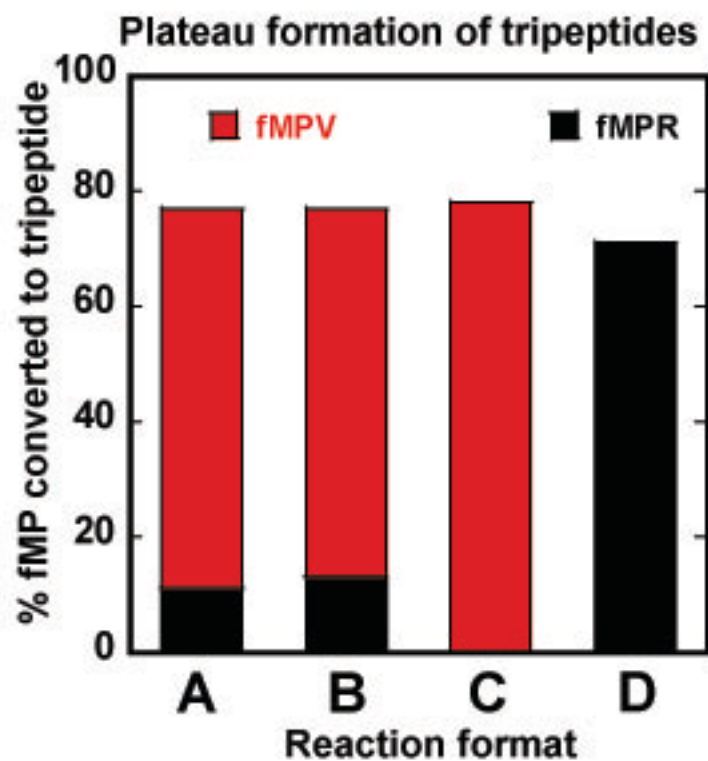


Figure S3. Analysis of the fractional conversion of fMP to fMPV or fMPR obtained under the four reaction schemes of Figure 5. The fractional conversion is calculated as the % of fMP that is converted to fMPV or fMPR. In contrast, the conversions shown in Figure 5 are calculated as the % of fMPV or fMPR in the sum of both tripeptides (fMPV + fMPR).