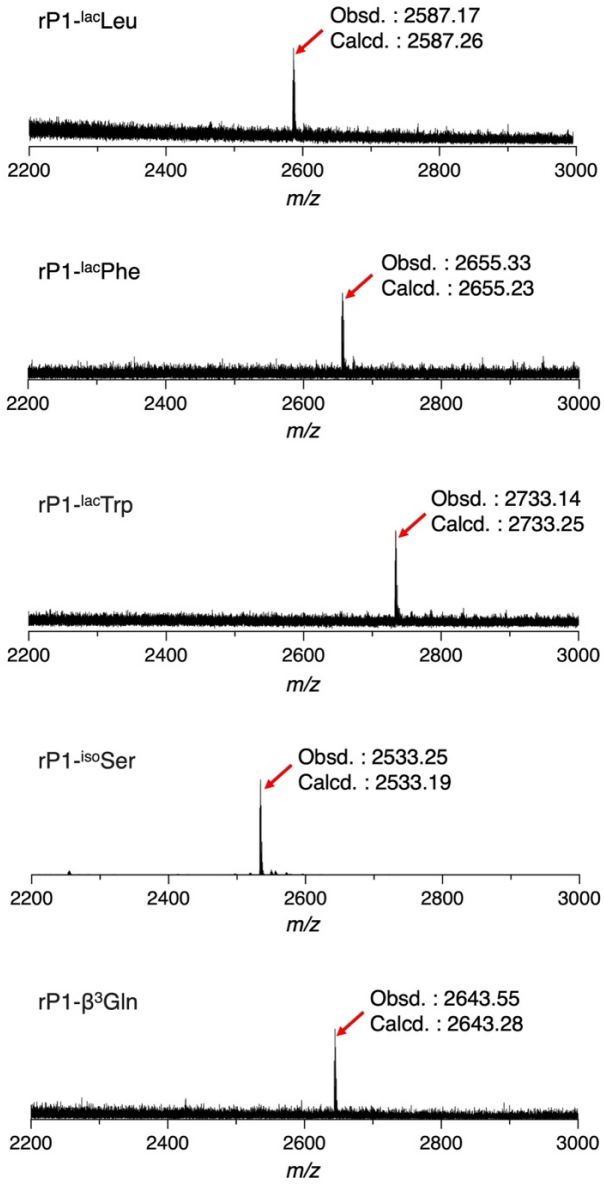
Supplemental Information for

EF-P Inhibits Ribosomal α-Hydroxy Acid Incorporation: Strategic tRNA Body Selection for Co-incorporating α-Hydroxy Acids and Nonproteinogenic Amino Acids into Depsipeptides

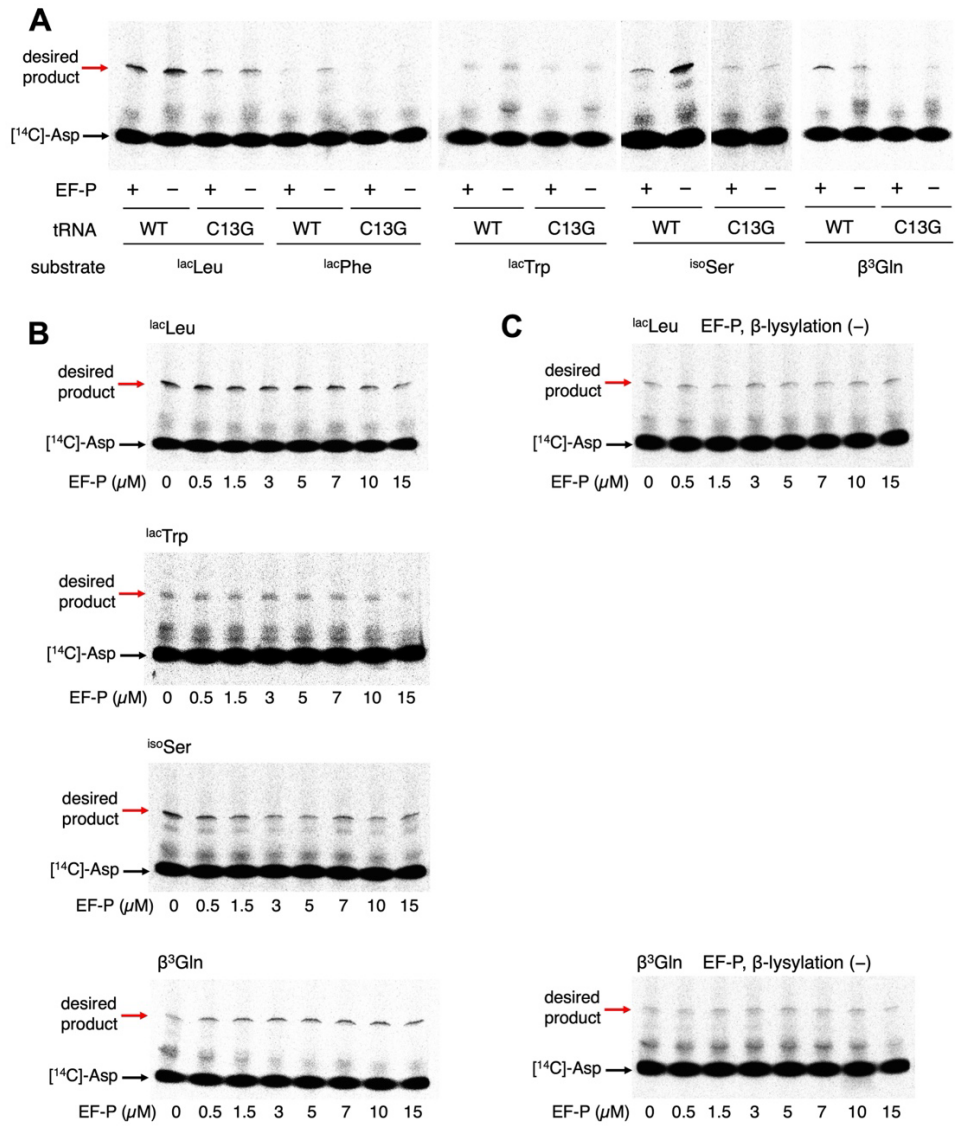
Takayuki Katoh[1]*

[1] Department of Chemistry, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033 (Japan)

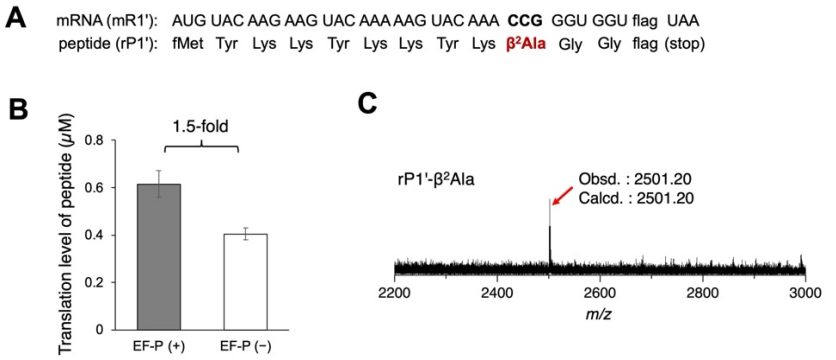
*E-mail: katoh@chem.s.u-tokyo.ac.jp



Supplemental Figure S1. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis of translated rP1 bearing α-hydroxy acids or β-amino acids. α-Hydroxy acids and β-amino acids were introduced using $tRNA^{Pro1}_{CGG}$ in the presence of 5 μM EF-P. Red arrows indicate peaks corresponding to the desired products. "Obsd." and "Calcd." denote observed and calculated *m/z* values, respectively.



Supplemental Figure S2. Tricine sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis of translated rP1 bearing α-hydroxy acids or β-amino acids. (A) Introduction of two consecutive α-hydroxy acids or β-amino acids at CCG codons using $tRNA^{Pro1}_{CGG}$ or $tRNA^{Pro1C13G}_{CGG}$ in the presence or absence of EF-P. (B,C) Titration of EF-P concentration for two consecutive incorporations of α-hydroxy acids or β-amino acids. EF-P bearing β-lysylation at Lys34 was used in B, whereas unmodified EF-P was used in C. Red arrows indicate bands corresponding to the desired products; black arrows indicate unreacted [^{14}C]-Asp. See also Fig. 3B and 3C for quantification of rP1.



Supplemental Figure S3. Ribosomal incorporation of β²Ala into a model peptide. (A) Sequence of model mRNA (mR1') and the corresponding peptide sequence (rP1'). (B) Translation levels of (rP1') in which β²Ala was introduced at a CCG codon using $tRNA^{Pro1}_{CGG}$ in the presence or absence of EF-P. Gray bars indicate EF-P (+) translation; white bars, EF-P (−) translation. The number above the bars represents the relative translation level, calculated as the ratio of EF-P (+) to EF-P (−). EF-P concentration in EF-P (+) translation was 5 μM. Error bars, s.d. (n = 3). (C) MALDI-TOF MS analysis of translated rP1'. β²Ala was introduced using $tRNA^{Pro1}_{CGG}$ in the presence of 5 μM EF-P. The red arrow indicates the peak corresponding to the desired product. "Obsd." and "Calcd." denote observed and calculated *m/z* values, respectively.