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Supplementary Materials for

EF-P Is Essential for Rapid Synthesis of Proteins Containing Consecutive Proline Residues

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Table S1

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Buffers and reagents

Buffer A: 50 mM Tris-HCl, pH 7.5 at 37°C, 70 mM NH_4Cl, 30 mM KCl and 7 mM $MgCl_2$; buffer B (HiFi): 50 mM Tris-HCl, pH 7.5 at 37°C, 70 mM NH_4Cl, 30 mM KCl, 3.5 mM $MgCl_2$, 0.5 mM spermidine, 8 mM putrescine and 2 mM DTT; buffer C: 50 mM Tris-HCl, pH 7.5 at 37°C, 70 mM NH_4Cl, 30 mM KCl, 1 mM spermidine, 16 mM putrescine and 4 mM DTT. Chemicals were from Roche Molecular Biochemicals, Sigma Aldrich, or Merck. Radioactive compounds were from Hartmann Analytic.

EF-P cloning, expression and purification

Genes coding for EF-P, YjeA, YjeK and YfcM were amplified from the *E. coli* genome (BL21(DE3) cells) and cloned into pET28a, adding an N-terminal His tag to EF-P as described (*5*). To overexpress EF-P alone or together with its modifying enzymes, the

following constructs were made: pET28efp, pET28efp/yjeA, pET28efp/yjeA/yjeK and pET28efp/yjeA/yjeK/yfcM.

EF-P was overexpressed in BL21(DE3). Cells were cultured in LB medium supplemented with kanamycin (30 µg/ml) at 37°C; expression was induced by the addition of IPTG (1 mM), and cultures were further grown for 3 h. Cells were harvested and pellets were resuspended in Protino buffer (20 mM Tris-HCl, pH 8.5, 300 mM NaCl, 5 mM 2-mercaptoethanol, 15% glycerol) with the addition of Complete Protease Inhibitor (Roche) and a trace of DNaseI. Cells were opened using an Emulsiflex apparatus, and the extract was centrifuged for 30 min at 300,000 g. The supernatant was applied to a Protino gravity-flow column (Macherey-Nagel) for affinity chromatography using the His tag. The column was washed with Protino buffer A and the protein was eluted with Protino buffer A containing 250 mM imidazole. The eluted protein was concentrated and the buffer was exchanged to 2 x buffer A by membrane filtration (Vivaspin 10,000); for storage, one volume of glycerol was added. The His tag was cleaved off with thrombin (GE Healthcare) in cleavage buffer (20 mM Tris-HCl, pH 8.4, 150 mM NaCl and 2.5 mM $CaCl_2$) with 2 units per 5000 pmol EF-P at room temperature overnight. The protein was concentrated by membrane filtration (Vivaspin 10,000). To remove the His tag and thrombin, EF-P was purified by FPLC on a HiTrapQ HP column using a 50 mM - 2 M NaCl gradient in 30 mM Tris-HCl, pH 7.5. EF-P containing fractions were pooled and concentrated and the buffer was exchanged to 2 x buffer A by membrane filtration (Vivaspin 10,000); for storage, one volume of glycerol was added. The concentration was determined by absorbance at 280

nm, assuming an extinction coefficient of 25,440 cm^{-1} M^{-1} (calculated on: www.biomol.net/en/tools/proteinextinction.htm).

Purification of native EF-P

Native EF-P was prepared as previously described (*28*) by pelleting EF-P in complex with ribosomes in the first step followed by a purification by FPLC. *E. coli* MRE600 cells were opened and ribosomes were pelleted as described (*29*), except that the NH_4Cl concentration in the opening buffer was 30 mM. EF-P was dissociated from ribosomes in high-salt buffer (10 mM Tris-HCl, pH 7.4, 1 M NH_4Cl, 10 mM $MgCl_2$, 3 mM 2-mercaptoethanol, 0.5 mM PefaBloc), ribosomes were pelleted at 300,000 g for 5 h, and the supernatant was dialyzed against dialysis buffer (25 mM HEPES, pH 7.4, 100 mM KCl, 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 0.5 mM PefaBloc). For ion-exchange chromatography on a HiTrap Q column (GE Healthcare), concentration of KCl was reduced to 50 mM by dilution with HiTrap Q buffer (25 mM HEPES, pH 7.4, 5% glycerol, 5 mM 2-mercaptoethanol). The protein was eluted by a gradient from 50 mM to 500 mM KCl. EF-P-containing fractions were identified by dot-blot using an anti-EF-P antibody (kindly provided by D. Görlich, Max Planck Institute for Biophysical Chemistry, Goettingen), pooled, and concentrated by membrane filtration (Vivaspin 10,000). EF-P was further purified by gel filtration on a HiLoad Superdex 75 column in buffer A. Fractions were analyzed by SDS-PAGE and western blotting; fractions containing EF-P were pooled and concentrated. For storage the buffer was exchanged to 2 x buffer A before adding one volume of glycerol.

Mass spectrometry

The identification and quantification of EF-P modifications was carried out by LC-MSMS. Protein samples (100 µg) were precipitated with acetone. Denaturation with Rapigest (Waters), reduction, alkylation, and trypsination were performed as described (*30*). Peptides were separated by reversed-phase Nanoflow chromatography on a Thermo Easy nLcII using chromatographic conditions as described (*30*). Eluting peptides were ionized by electrospray ionization (ESI) on a Q Exactive mass spectrometer (Thermo Fisher Scientific) and analyzed in the data-dependent mode. MS scans were acquired in the range *m/z* 350-1600 with a resolution of 70,000 and twelve peaks of the highest intensity were selected for HCD MS/MS fragmentation. The dynamic exclusion was 20 s. Singly-charged ions and ions with unrecognized charge state were excluded. Peptides were quantified by integrating over the corresponding extracted ion chromatograms (XIC). XICs were generated with a mass tolerance of 10 ppm using the Thermo Excalibur software (version 2.2 SPI48). The overall signal intensity in different runs was corrected by normalization, using as internal reference two razor peptides (VPLFVQIGEVIK and GDTAGTGGKPATLSTGAVVK) that were well observable and chemically stable. The charge state of highest intensity (z=3 for unmodified EF-P peptide and z=5 for lysinylated and lysinylated/hydroxylated EF-P peptide) and the most prominent peak in the isotope distribution of the respective peptides was selected for quantification by integration. Quantification based on the monoisotopic mass led to similar results, albeit at poor signal-to-noise ratios for low-abundance peptides. Spectra shown in **fig. S3B,C** show the corresponding peptides with z=4 that were less

populated but yielded better spectra then those with *z*=5. Because the degree of Met oxidation was reproducibly small, only the unoxidized peptides were quantified.

mRNA constructs

mRNAs used in **Fig. 1** were purchased from IBA. The genes coding for AmiB, PrmC, TonB, Rz1, and YafD were amplified from the genomic DNA of *E. coli* BL21(DE3) by colony PCR, digested with *NdeI* and *Xho1* (New England Biolabs) and ligated into the pET24a vector (Novagen). Templates for transcription were generated by PCR amplification of the respective gene (including the T7 promoter and the Shine-Dalgarno sequence of the pET24 5´UTR) coding for AmiB (1-159), PrmC (1-75), TonB (1- 239), Rz1 (1-62) and YafD (1-75). For peptide markers, mRNA templates of desired length were prepared. Transcription was performed in transcription buffer (40 mM Tris-HCl, pH 7.5, 15 mM $MgCl_2$, 2 mM spermidine, 10 mM NaCl, 10 mM DTT, 3 mM NTPs each, 5 mM GMP, 10% (v/v) DNA template, 5 u/ml pyrophosphatase, 1.5% (v/v) RiboLock RNase inhibitor (Fermentas) and 0.8% (v/v) T7 RNA-polymerase) for 3 h at 37°C. The mRNAs were purified from the crude transcription mixture using the RNeasy Midi Kit (Qiagen) according to the manufacturer's protocol.

Ribosomes, factors and tRNAs

Ribosomes from *E. coli* MRE600, initiation factors (IF1, IF2, IF3), EF-Tu, EF-G, and some individual tRNAs (fMet-tRNA^{fMet}, Phe-tRNA^{Phe}, Lys-tRNA^{Lys}, Gly-tRNA^{Gly}, Trp-tRNA^{Trp},

Val-tRNA^{Val}, Arg-tRNA^{Arg}, Glu-tRNA^{Glu}) were prepared as described (*31*). Other tRNAs (Pro-tRNA^{Pro}, Asp-tRNA^{Asp}, Gln-tRNA^{Gln}) were prepared as follows. Total tRNA from *E. coli* was aminoacylated with the respective ^{14}C-labeled amino acid (*32*). After phenol extraction and ethanol precipitation, aminoacyl-tRNA was enriched by high performance liquid chromatography (HPLC) on a Lichrospher WP 300 column (Merck) using a gradient of 0-15% ethanol in 20 mM ammonium acetate, pH 5.0, 10 mM magnesium acetate, 400 mM NaCl. After ethanol precipitation, aminoacyl-tRNA was dissolved in water, and added to EF-Tu·GTP to form ternary complex which was purified by FPLC gel filtration on two Superdex 75 columns run in tandem. Fractions containing ternary complex were extracted by phenol and the aminoacyl-tRNA was isolated by ethanol precipitation.

Ribosome complexes

Initiation complexes were prepared by incubating 70S ribosomes (1 µM) with a 3-fold excess of mRNA, IF1, IF2, IF3 (1.5 µM each), fMet-tRNA^{fMet} (1.5 µM; amino acid labeled with ^{14}C/^{3}H or with Bodipy FL when translation products were to be visualized on SDS-PAGE) and GTP (1 mM) in buffer A for 30 min (*14*). For experiments presented in **Figs. 1 and4** as well as **figs. S1 and S2A**, initiation complexes were purified by centrifugation through 400 µl sucrose cushions (1.1 M in buffer A) at 260,000 g for 2 h. Pellets were dissolved in buffer A and complexes stored at -80°C. In all other cases, when initiation complexes were used without prior purification, complexes were diluted with buffer C to yield buffer B.

Ternary complexes, EF-Tu·GTP·aminoacyl-tRNA, were prepared by incubating EF-Tu (50 µM, containing a C-terminal His tag) with pyruvate kinase (0.1 mg/ml), phosphoenolpyruvate (3 mM) and GTP (1 mM) for 20 min at 37°C in buffer A or B. Aminoacyl-tRNA was added in a 1:2 ratio to EF-Tu and the ternary complex was either purified by gel filtration on a Superdex 75 tandem column (*31*) or used without prior purification.

Posttranslocation complexes used in the experiment of **Fig. 1A,B** were formed by mixing initiation complexes with a 1.5-fold excess of the corresponding unpurified ternary complex. Translocation was initiated by the addition of EF-G (1/10 molar ratio to the ribosome). Posttranslocation complexes were purified by centrifugation through a sucrose cushion as described above for initiation complexes. For the experiments shown in **fig. S2B,** initiation complexes were prepared with mRNA coding for mutant PrmC containing the PPG sequence and were used without prior purification; the ternary complex was prepared from total aa-tRNA labeled with [^{14}C]Pro and [^{3}H]Gly, EF-Tu, and GTP and purified as described above. Initiation complexes (0.2 µM) were mixed with ternary complexes (9.6 µM) in buffer B and incubated for 3 min at 37°C. Ribosome-nascent-chain complexes were purified from ternary complexes, tRNAs, EF-G, and GTP by gel filtration (Biosuite 450 8µm HR SEC, Waters, at a flow of 0.8 ml/min in buffer B). [^{14}C]Pro and [^{3}H]Gly eluting in the ribosome peak were quantified by double-label scintillation counting. Fractions containing ribosome-nascent chain complexes were pooled, and the samples were hydrolyzed in 0.5 M KOH for 30 min at 37°C and neutralized with acetic acid. Translation products and amino acids were separated by reversed phase HPLC (Nucleosil 300-5 C4,

Macherey Nagel) applying a gradient from 0 to 65% acetonitrile/0.1% TFA in 20 min. [^{14}C]Pro and [^{3}H]Gly were quantified by scintillation counting.

Quench-flow experiments

The Pmn reaction (**Fig. 1A**) was performed in buffer A at 37°C as described (*14*) with purified ribosome complexes and subsaturating concentrations of Pmn, which ensures the maximum sensitivity to measure effects on both affinity and catalysis. In the reactions with dipeptidyl-tRNAs, the final Pmn concentration was 1 mM, whereas with fMet-tRNA^{fMet} the Pmn concentration was reduced to 100 µM, because the K_M of the reaction, 300 µM, is lower than with dipeptidyl-tRNAs (*14*). Reaction time courses were measured in a quench-flow apparatus (KinTek Laboratories, Inc.) by mixing equal volumes (14 µl) each of the ribosome complex (0.15 µM) and Pmn.

Rates of di- and tripeptide formation (**Fig. 1B)** were determined using purified initiation (fM) or posttranslocation (fMP) complexes (0.2 µM) and adding unpurified ternary complexes at saturating concentrations (10 µM) in buffer B at 37°C. Reactions were quenched with KOH (0.5 M), samples hydrolyzed for 30 min at 37°C, neutralized with acidic acid, and analyzed by reversed phase HPLC (LiChrospher 100 RP-8 or Chromolith RP8 100-4.6 mm column, Merck) using a 0-65% acetonitrile gradient in 0.1% TFA. Products and educts were quantified by double-label scintillation counting. Where appropriate in the experiments of **Figs. 1A,B**, EF-P (overexpressed, lysinylated; 3 µM) was present in both syringes. The experiments of **Fig. 1C** were performed using purified initiation complexes (0.2 µM), unpurified ternary complexes (2 µM each as specified by

the mRNA sequence), EF-G (1 µM), with or without EF-P (native, lysinylated/hydroxylated; 3 µM) in buffer B at 37°C. The experiments of **Fig. 4** were carried out in the same way, except that overexpressed lysinylated/hydroxylated EF-P was used if not stated otherwise. Rate constants were evaluated by exponential fitting using GraphPad or Scientist software. Data shown are the mean of three independent experiments; error bars represent standard deviations.

In-vitro translation

For *in-vitro* translation, initiation complexes and ternary complexes were prepared in buffer A and mixed with an equal amount of buffer C to reach buffer B. To compensate for Mg^{2+} binding to GTP and PEP (*33*), the $MgCl_2$ concentration was increased to 3.5 mM free $MgCl_2$. Ternary complex (40 µM for sequences coding for 75 amino acids) and EF-G (2 µM) were rapidly mixed with initiation complex (20 nM). EF-P (3 µM) was added both to the factor and the ribosome mix; the experiments were carried out with either native or overexpressed lysinylated/hydroxylated factor. The concentrations of ternary complex were increased proportionally to the length of the protein to be translated. For exceptionally proline-rich proteins (TonB, AmiB, Rz1, and YafD), the total aminoacyl-tRNA was supplemented with Pro-$tRNA^{Pro}$ (10 equivalents of Pro-$tRNA^{Pro}$ per encoded proline). Translation was carried out at 37° and the reaction stopped by addition of 1/10 volume of 2 M NaOH. The specific peptide markers were synthesized in the same way in the presence of EF-P (3 µM) for 10 min at 37°C. After hydrolysis for 30 min at 37°C, the samples were neutralized by addition of 1/10 volume 2 M HEPES pH 7, incubated in loading buffer (50

mM Tris-HCl pH 6.8, 12% (w/v) glycerol, 2% 2-mercaptoethanol, 4% SDS) for 30 min at 40°C and loaded onto the 10-20% or 16.5 % Tris/Tricine SDS gels (Criterion, BIO-RAD) and PAGE was carried out using commencial Tris/Tricine buffer (BIO-RAD). Gels were incubated in water for 5 min and scanned on a FLA-9000 fluorescence imager (Fuji) at 50 µm resolution. Bodipy fluorescence was excited at 473 nm and monitored after passing a LPB (510LP) cut-off filter.

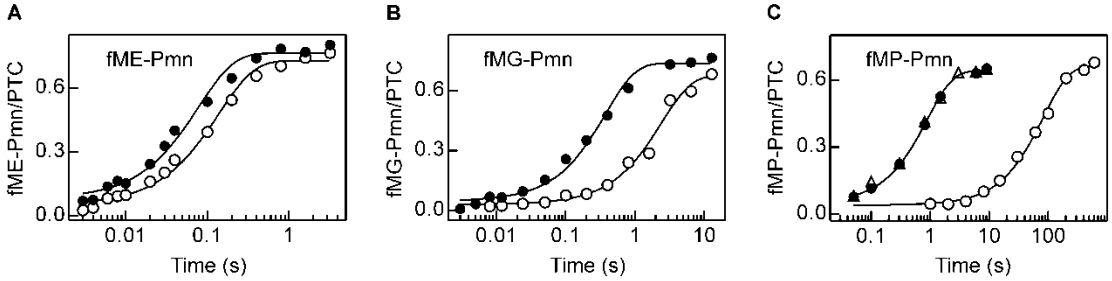

Fig. S1. Time courses of fMetX-Pmn formation. (**A**) fMetAsp-Pmn. (**B**) fMetGly-Pmn. (**C**) fMetPro-Pmn. Reactions were carried out in the absence (open circles) or presence of overexpressed lysinylated EF-P (closed circles) or lysinylated/hydroxylated EF-P (open triangles). Ribosomes carrying dipeptidyl-tRNA as indicated were mixed with Pmn in the quench-flow apparatus. At the indicated times, the reaction was quenched and the products analyzed by HPLC (Materials and Methods).

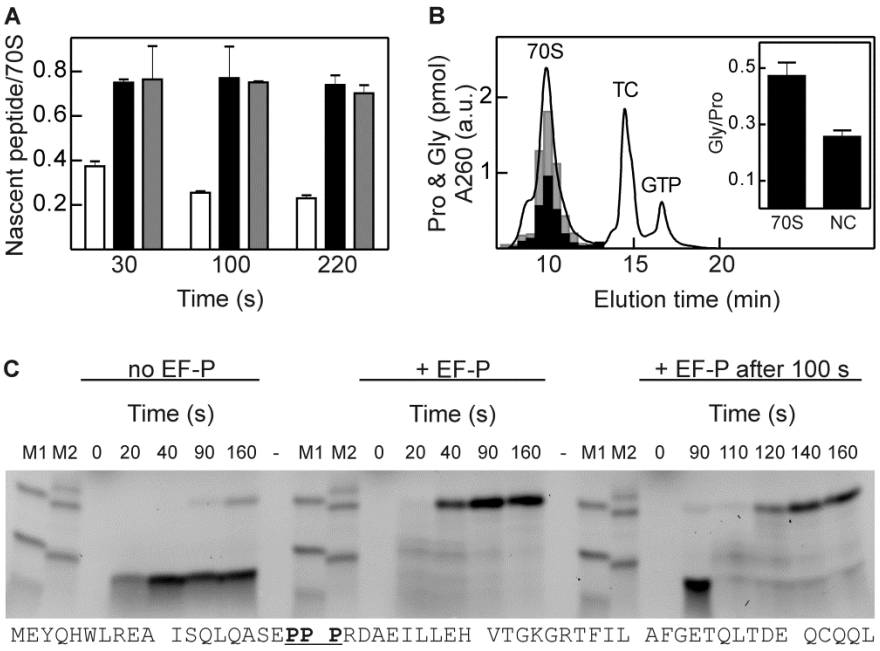

Fig. S2. Binding of peptidyl-tRNAs to stalled ribosomes. (**A**). Retention of short peptidyl-tRNAs resulting from translation of fMPPGF in the absence (white bars) or presence (black bars) of EF-P (3 µM) or of fMFFF in the absence of EF-P (gray bars). The reaction was carried out as described for **Fig. 1C**. Ribosome-bound peptidyl-tRNA was isolated by nitrocellulose filtration and quantified by radioactivity counting. Peptides were labeled with [^{14}C]Met and [^{14}C]Pro (fMPPGF) or [^{3}H]Met and [^{14}C]Phe (fMFFF). (**B**) Retention of [^{14}C]Pro (gray bars) and [^{3}H]Gly (black bars) in ribosome-nascent-chain complexes (identified in the elution profile from a gel filtration column by optical density at A_{260}; black line) stalled on the PPG sequence in PrmC in the absence of EF-P. Inset: Gly/Pro stoichiometry in the ribosome-nascent chain complex (70S; if Gly and both Pro

residues are bound, a ratio of 0.5 is expected) or incorporated into the nascent chain (NC; a ratio should increase from 0 to 0.5 with the portion of Gly incorporated into peptide). (**C**) Rescue of PrmC translation after addition of EF-P. Left panel, ribosome stalling on the PPP sequence in the absence of EF-P. Middle panel, translation of full-length product in the presence of EF-P. Right panel, resuming of translation upon addition of EF-P (3 μM) to stalled ribosome complexes after 100 s of pausing in the absence of the factor.

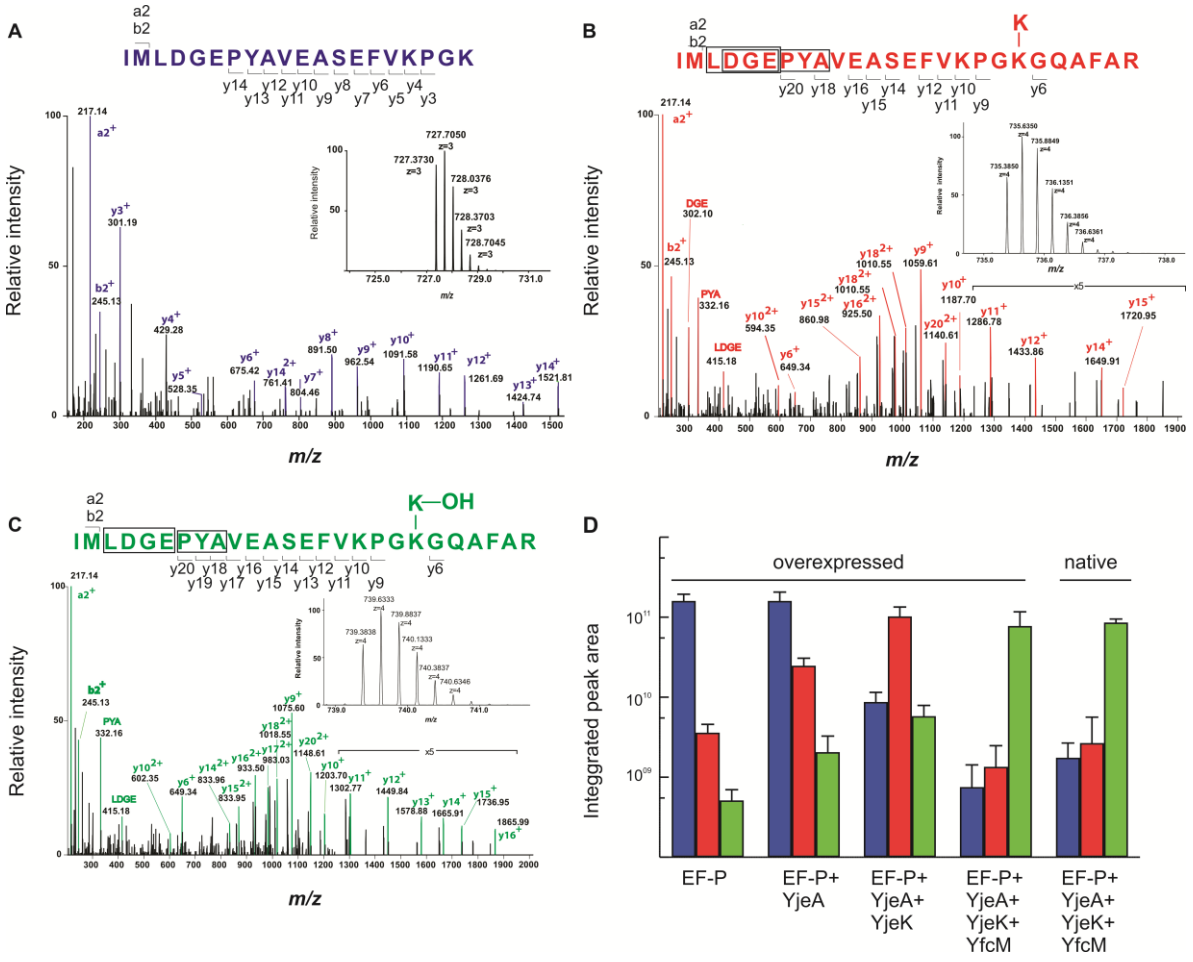

Fig. S3: Analysis of EF-P modifications by mass spectrometry. (A) MS/MS spectrum of the unmodified peptide (amino acids 15-34). The y-type product ion series clearly identify the sequence of the peptide lacking the modification. Inset: MS spectrum (*m/z*= 727.3696) of the intact and unmodified peptide (*z*=3). (**B**) MS/MS spectrum of the lysinylated peptide (amino acids 15-40). Inset: MS spectrum (*m/z*= 735.3837) of the intact and lysinylated quadruply charged peptide (*z*=4). (**C**) MS/MS spectrum of the lysinylated and hydroxylated peptide (amino acids 15-40). Inset: MS spectrum (*m/z*= 739.3825) of the intact, lysinylated

and hydroxylated quadruply charged peptide (*z*=4). (**D**) Relative quantification of the modifications in different EF-P preparations. The unmodified (blue), the lysinylated (red) and the lysinylated/hydroxylated (green) peptides were quantified by integration of their respective extracted ion chromatograms (XIC). Error bars represent standard deviation of three technical replicates. For the quantification, the charge states with the maximum signal intensity were used, *z*=3 for the unmodified EF-P and *z*=5 for the lysinylated and lysinylated/hydroxylated EF-P.

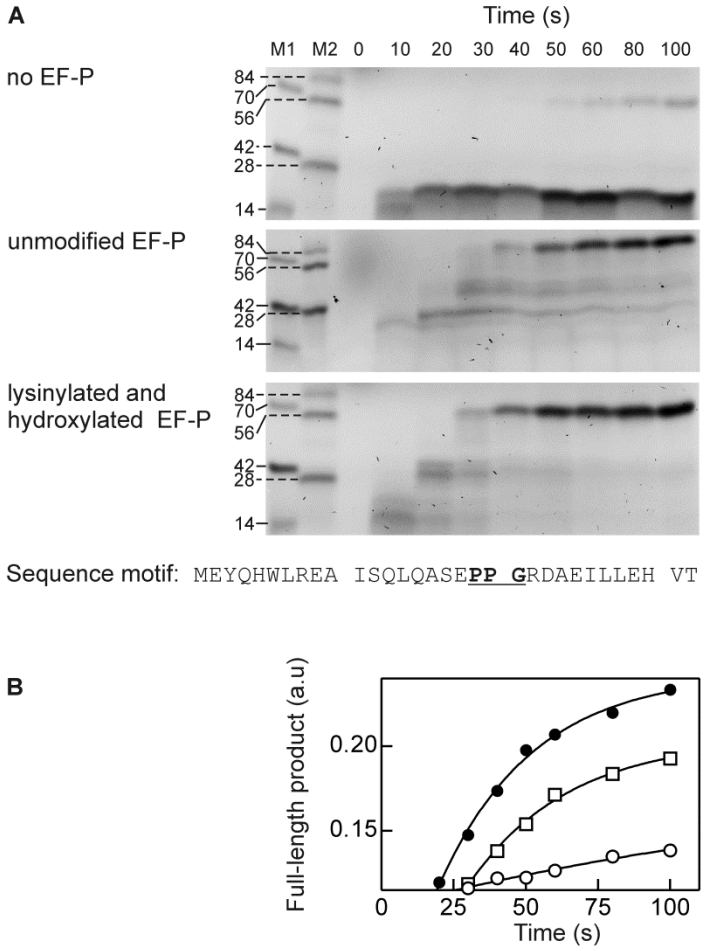

Fig. S4. Effect of EF-P modification on the translation of mutant PrmC containing a PPG sequence. (**A**) Time courses of translation visualized by analysis of translation products by SDS-PAGE and fluorescence imaging. (**B**) Quantification of the translation kinetics of full-length product formation in the absence of EF-P (open circles), with unmodified EF-P (open squares) or overexpressed lysinylated/hydroxylated EF-P (closed circles). The density of the product band is normalized to the total density in the respective lane and presented in arbitrary units (a.u.).

Table S1. Proteins containing consecutive prolines. The data were extracted from the *E. coli* (K12) genome through the uniprot database.

Sequence	Protein name
PPPPPPPP	1.AMIB_ECOLI N-acetylmuramoyl-L-alanine amidase AmiB
PPPPP	1. RZOR_ECOLI Outer membrane lipoprotein Rz1 from lambdoid prophage 2.YAAX_ECOLI Uncharacterized protein yaa
PPPP	1. C56H_ECOLI Cytochrome b561 homolog 1 YODB 2. CLS_ECOLI Cardiolipin synthase 3. ENTS_ECOLI Enterobactin exporter EntS 4. MTLR_ECOLI Mannitol operon repressor 5. YCGL_ECOLI UPF0745 protein YcgL 6. YDEI_ECOLI Uncharacterized protein ydeI
PPP	1. ACEK_ECOLI Isocitrate dehydrogenase kinase/phosphatase 2. ADRA_ECOLI Probable diguanylate cyclase AdrA 3. ADRB_ECOLI Putative cyclic-di-GMP phosphodiesterase AdrB 4. AGP_ECOLI Glucose-1-phosphatase 5. BCSB_ECOLI Cyclic di-GMP-binding protein 6. C56I_ECOLI Cytochrome b561 homolog 2 7. CAPP_ECOLI Phosphoenolpyruvate carboxylase 8. CATE_ECOLI Catalase HPII 9. CREC_ECOLI Sensor protein CreC 10. CYOB_ECOLI Ubiquinol oxidase subunit 1 11. DPPF_ECOLI Dipeptide transport ATP-binding protein DppF 12. ENTF_ECOLI Enterobactin synthase component F 13. ENVZ_ECOLI Osmolarity sensor protein EnvZ 14. EUTL_ECOLI Ethanolamine utilization protein EutL 15. EX5B_ECOLI Exodeoxyribonuclease V beta 16. FLHC_ECOLI Flagellar transcriptional regulator FlhC 17. FOCB_ECOLI Probable formate transporter 2 18. FLK_ECOLI Flagellar regulator flk 19. FTSK_ECOLI DNA translocase FtsK 20. GFCC_ECOLI Uncharacterized protein gfcC 21. GGT_ECOLI Gamma-glutamyltranspeptidase 22. GLPR_ECOLI Glycerol-3-phosphate regulon repressor

23. GLTB_ECOLI Glutamate synthase [NADPH] large chain
24. GNTX_ECOLI Protein GntX
25. GPR_ECOLI L-glyceraldehyde 3-phosphate reductase
26.HCAD_ECOLI 3-phenylpropionate/cinnamic acid dioxygenase ferredoxin--NAD(+) reductase component
27. HISX_ECOLI Histidinol dehydrogenase
28. HOFM_ECOLI Putative DNA utilization protein HofM
29. HOLB_ECOLI DNA polymerase III subunit delta
30. HYAF_ECOLI Hydrogenase-1 operon protein hyaF
31. HYFR_ECOLI Hydrogenase-4 transcriptional activator
32. K6PF2_ECOLI 6-phosphofructokinase isozyme 2
33. LEPA_ECOLI Elongation factor 4
34. LIGT_ECOLI 2'-5'-RNA ligase OS=Escherichia coli
35. LSRC_ECOLI Autoinducer 2 import system permease protein lsrC
36. MALG_ECOLI Maltose transport system permease protein malG
37. MEPA_ECOLI Penicillin-insensitive murein endopeptidase
38. NLPC_ECOLI Probable endopeptidase NlpC
39. NLPD_ECOLI Murein hydrolase activator NlpD
40. NUDC_ECOLI NADH pyrophosphatase
41. NUOCD_ECOLI NADH-quinone oxidoreductase subunit C/D
42. PAT_ECOLI Putrescine aminotransferase
43. PBPA_ECOLI Penicillin-binding protein 1A
44. PHOR_ECOLI Phosphate regulon sensor protein phoR
45. PSTA_ECOLI Phosphate transport system permease protein pstA
46. QOR1_ECOLI Quinone oxidoreductase 1
47. RECG_ECOLI ATP-dependent DNA helicase recG
48. RNB_ECOLI Exoribonuclease 2
49. RSMA_ECOLI Ribosomal RNA small subunit methyltransferase A
50. RUTD_ECOLI Putative aminoacrylate hydrolase
51. RUTG_ECOLI Putative pyrimidine permease
52. RZOD_ECOLI Outer membrane lipoprotein Rz1 from lambdoid prophage DLP12
53. RZOR_ECOLI Outer membrane lipoprotein Rz1 from lambdoid prophage Rac
54. SDAC_ECOLI Serine transporter
55. SFGH2_ECOLI S-formylglutathione hydrolase yeiG
56. SLYX_ECOLI Protein SlyX
57. SYV_ECOLI Valine--tRNA ligase
58. TAMA_ECOLI Translocation and assembly module TamA
59. TAUC_ECOLI Taurine transport system permease protein tauC
60. TONB_ECOLI Protein tonB
61. UVRB_ECOLI UvrABC system protein B
62. YACH_ECOLI Uncharacterized protein yacH
63. YAEI_ECOLI Phosphodiesterase yaeI
64. YAFZ_ECOLI UPF0380 protein yafZ

65. YAGS_ECOLI Putative xanthine dehydrogenase YagS FAD-binding subunit
66. YCGG_ECOLI Uncharacterized protein YcgG
67. YCGL_ECOLI UPF0745 protein YcgL
68. YCGR_ECOLI Flagellar brake protein YcgR
69. YCHG_ECOLI Putative uncharacterized protein ychG
70. YDCO_ECOLI Inner membrane protein ydcO
71. YDEI_ECOLI Uncharacterized protein ydeI
72. YDIK_ECOLI UPF0118 inner membrane protein ydiK
73. YEEJ_ECOLI Uncharacterized protein yeeJ
74. YEGI_ECOLI Uncharacterized protein yegI
75. YFBK_ECOLI Uncharacterized protein yfbK
76. YFCO_ECOLI Uncharacterized protein yfcO
77. YFCR_ECOLI Uncharacterized protein yfcR
78. YFDK_ECOLI Uncharacterized protein yfdK
79. YFJQ_ECOLI UPF0380 protein yfjQ
80. YHBW_ECOLI Uncharacterized protein yhbW
81. YHFW_ECOLI Uncharacterized protein yhfW
82. YIDI_ECOLI Inner membrane protein yidI
83. YIFB_ECOLI Uncharacterized protein YifB
84. YIFL_ECOLI Uncharacterized lipoprotein yifL
85. YJBG_ECOLI Uncharacterized protein yjbG
86. YJDA_ECOLI Uncharacterized protein yjdA
87. YPDI_ECOLI Uncharacterized lipoprotein ypdI

PPG:

1. ACCD_ECOLI Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta
2. ACON1_ECOLI Aconitate hydratase 1
3. ACON2_ECOLI Aconitate hydratase 2
4. ACPT_ECOLI 4'-phosphopantetheinyl transferase AcpT
5. ADIA_ECOLI Biodegradative arginine decarboxylase
6. ADRA_ECOLI Probable diguanylate cyclase AdrA
7. ALDB_ECOLI Aldehyde dehydrogenase B
8. AMPD_ECOLI 1,6-anhydro-N-acetylmuramyl-L-alanine amidase AmpD
9. AMPH_ECOLI D-alanyl-D-alanine-carboxypeptidase/endopeptidase
10. ARAC_ECOLI Arabinose operon regulatory protein
11. ARCM_ECOLI Carbamate kinase-like protein YahI
12. AROC_ECOLI Chorismate synthase
13. ASTD_ECOLI N-succinylglutamate 5-semialdehyde dehydrogenase
14. ATPA_ECOLI ATP synthase subunit alpha
15. ATPB_ECOLI ATP synthase subunit beta
16. AZUC_ECOLI Uncharacterized protein AzuC
17. BCSB_ECOLI Cyclic di-GMP-binding protein
18. BGLR_ECOLI Beta-glucuronidase
19. C56I_ECOLI Cytochrome b561 homolog 2

20. CAAL_ECOLI Carboxylate-amine ligase YbdK
21. CATE_ECOLI Catalase HPII
22. CBPA_ECOLI Curved DNA-binding protein
23. CHEB_ECOLI Chemotaxis response regulator protein-glutamate methylesterase
24. CINAL_ECOLI CinA-like protein
25. CLPA_ECOLI ATP-dependent Clp protease ATP-binding subunit ClpA
26. CLPB_ECOLI Chaperone protein ClpB
27. CPTA_ECOLI Phosphoethanolamine transferase CptA
28. CPXA_ECOLI Sensor protein CpxA
29. CURA_ECOLI NADPH-dependent curcumin reductase
30. CYAA_ECOLI Adenylate cyclase
31. CYSQ_ECOLI 3'(2'),5'-bisphosphate nucleotidase CysQ
32. DAPE_ECOLI Succinyl-diaminopimelate desuccinylase
33. DCLZ_ECOLI Lysine decarboxylase
34. DCOR_ECOLI Ornithine decarboxylase
35. DCOS_ECOLI Ornithine decarboxylase
36. DINF_ECOLI DNA-damage-inducible protein
37. DMSA_ECOLI Dimethyl sulfoxide reductase DmsA
38. DNAT_ECOLI Primosomal protein 1
39. DPIB_ECOLI Sensor histidine kinase DpiB
40. DPPF_ECOLI Dipeptide transport ATP-binding protein DppF
41. ENTF_ECOLI Enterobactin synthase
42. ETK_ECOLI Tyrosine-protein kinase etk
43. EUTJ_ECOLI Ethanolamine utilization protein eutJ
44. EX5C_ECOLI Exodeoxyribonuclease V gamma chain
45. EXOX_ECOLI Exodeoxyribonuclease 10
46. FADK_ECOLI Short-chain-fatty-acid--CoA ligase
47. FADR_ECOLI Fatty acid metabolism regulator protein
48. FDOG_ECOLI Formate dehydrogenase-O major subunit
49. FIMD_ECOLI Outer membrane usher protein fimD
50. FLIF_ECOLI Flagellar M-ring protein
51. FLK_ECOLI Flagellar regulator flk
52. FTSH_ECOLI ATP-dependent zincmetalloprotease FtsH
53. GALF_ECOLI UTP--glucose-1-phosphateridylyltransferase
54. GALU_ECOLI UTP--glucose-1-phosphateuridylyltransferase
55. GFCC_ECOLI Uncharacterized protein gfcC
56. GPR_ECOLI L-glyceraldehyde 3-phosphate reductase
57. HOFM_ECOLI Putative DNA utilization protein HofM
58. HSLU_ECOLI ATP-dependent protease ATPase subunit HslU
59. HTRE_ECOLI Outer membrane usher protein htrE
60. HYPF_ECOLI Carbamoyltransferase hypF
61. ICIA_ECOLI Chromosome initiation inhibitor
62. ILVB_ECOLI Acetolactate synthase isozyme
63. ILVG_ECOLI Acetolactate synthase isozyme 2 large subunit

64. INTE_ECOLI Prophage lambda integrase
65. IRAM_ECOLI Anti-adapter protein iraM
66. K6PF2_ECOLI 6-phosphofructokinase isozyme 2
67. KDPD_ECOLI Sensor protein KdpD
68. LCFA_ECOLI Long-chain-fatty-acid--CoA ligase
69. LDCI_ECOLI Lysine decarboxylase
70. LEP_ECOLI Signal peptidase
72. LIGT_ECOLI 2'-5'-RNA ligase
73. LON_ECOLI Lon protease
74. LSRK_ECOLI Autoinducer 2 kinase LsrK
75. LYSR_ECOLI Transcriptional activator protein lysR
76. MCRB_ECOLI 5-methylcytosine-specific restriction enzyme B
77. METF_ECOLI 5,10-methylenetetrahydrofolate reductase
78. MLTA_ECOLI Membrane-bound lytic murein transglycosylase A
79. MNME_ECOLI tRNA modification GTPase MnmE
80. MPAA_ECOLI Protein mpaA
81. MRP_ECOLI Protein mrp
82. MURD_ECOLI UDP-N-acetylmuramoylalanine--D-glutamate ligase
83. MUTL_ECOLI DNA mismatch repair protein mutL
84. NAPG_ECOLI Ferredoxin-type protein napG
85. NARZ_ECOLI Respiratory nitrate reductase 2 alpha chain
86. NUDC_ECOLI NADH pyrophosphatase
87. NUPX_ECOLI Nucleoside permease nupX
88. PARC_ECOLI DNA topoisomerase 4 subunit A
90. PARC_ECOLI DNA topoisomerase 4 subunit A
91. PAT_ECOLI Putrescine aminotransferase
92. PBPA_ECOLI Penicillin-binding protein 1A
93. PHNI_ECOLI Protein phnI
94. PHOR_ECOLI Phosphate regulon sensor protein phoR
95. PHR_ECOLI Deoxyribodipyrimidine photo-lyase
96. POTH_ECOLI Putrescine transport system permease protein
97. POTD_ECOLI Spermidine/putrescine-binding periplasmic protein
98. PPA_ECOLI Periplasmic AppA protein
99. PRIM_ECOLI DNA primase
100. PRPE_ECOLI Propionate--CoA ligase
101. PSTC_ECOLI Phosphate transport system permease protein pstC
102. PSUT_ECOLI Putative pseudouridine transporter
103. PTH_ECOLI Peptidyl-tRNA hydrolase
104. PTNAB_ECOLI PTS system mannose-specific EIIAB component
105. PUTA_ECOLI Bifunctional protein putA
106. QSEC_ECOLI Sensor protein qseC
107. RARA_ECOLI Replication-associated recombination protein A
108. RAVA_ECOLI ATPase ravA
109. RBN_ECOLI Ribonuclease BN
110. RECG_ECOLI ATP-dependent DNA helicase recG

111. RFAG_ECOLI Lipopolysaccharide core biosynthesis protein rfaG
112. RNFE_ECOLI Electron transport complex protein RnfE
113. RRMF_ECOLI Probable rRNA maturation factor YbeY
114. RSEC_ECOLI Sigma-E factor regulatory protein RseC
115. RUVB_ECOLI Holliday junction ATP-dependent DNA helicase RuvB
116. SAPC_ECOLI Peptide transport system permease protein sapC
117. SFMF_ECOLI Fimbrial-like protein sfmF
118. SFMH_ECOLI Protein sfmH
119. SPOT_ECOLI Bifunctional (p)ppGpp synthase/hydrolase SpoT
120. SRP54_ECOLI Signal recognition particle protein
122. SURE_ECOLI 5'/3'-nucleotidase SurE
123. SYA_ECOLI Alanine--tRNA ligase
124. TMCA_ECOLI tRNA(Met) cytidine acetyltransferase TmcA
125. TRKA_ECOLI Trk system potassium uptake protein trkA
126. UHPB_ECOLI Sensor protein uhpB
127. WCAM_ECOLI Colanic acid biosynthesis protein wcaM
128. Y1142_ECOLI Putative uncharacterized protein b1142
129. YAFD_ECOLI UPF0294 protein yafD
130. YAGS_ECOLI Putative xanthine dehydrogenase YagS FAD-binding subunit
132. YAHG_ECOLI Uncharacterized protein yahG
133. YBAB_ECOLI UPF0133 protein YbaB
134. YBBP_ECOLI Uncharacterized ABC transporter permease ybbP
135. YBFO_ECOLI Putative uncharacterized protein ybfO
136. YBGQ_ECOLI Uncharacterized outer membrane usher protein ybgQ
137. YBIC_ECOLI Uncharacterized oxidoreductase ybiC
138. YBIO_ECOLI Uncharacterized MscS family protein YbiO
139. YBIU_ECOLI Uncharacterized protein ybiU
140. YBJD_ECOLI Uncharacterized protein ybjD
141. YCEG_ECOLI UPF0755 protein yceG
142. YCFD_ECOLI Uncharacterized protein ycfD
143. YCFS_ECOLI Probable L,D-transpeptidase YcfS
144. YCFZ_ECOLI Inner membrane protein ycfZ
145. YCGY_ECOLI Uncharacterized protein ycgY
146. YDAU_ECOLI Uncharacterized protein ydaU
147. YDCU_ECOLI Inner membrane ABC transporter permease protein ydcU
148. YDCR_ECOLI Uncharacterized HTH-type transcriptional regulator YdcR
149. YDDK_ECOLI Putative uncharacterized protein yddK
150. YDHK_ECOLI Uncharacterized transporter YdhK
151. YDHV_ECOLI Uncharacterized oxidoreductase YdhV
152. YEBT_ECOLI Uncharacterized protein yebT
153. YEEJ_ECOLI Uncharacterized protein yeeJ
154. YEHB_ECOLI Uncharacterized outer membrane usher protein yehB

155. YEHL_ECOLI Uncharacterized protein yehL
156. YFDE_ECOLI Uncharacterized protein YfdE
157. YFJK_ECOLI Uncharacterized protein yfjK
158. YGAQ_ECOLI Putative uncharacterized protein ygaQ
159. YGBN_ECOLI Inner membrane permease
160. YGCE_ECOLI Uncharacterized sugar kinase YgcE
161. YGCQ_ECOLI Putative electron transfer flavoprotein subunit ygcQ
162. YGGC_ECOLI Uncharacterized protein yggC
163. YGHS_ECOLI Uncharacterized ATP-binding protein yghS
164. YHJC_ECOLI Uncharacterized HTH-type transcriptional regulator YhjC
165. YIDD_ECOLI Putative membrane protein insertion efficiency factor
166. YIEL_ECOLI Uncharacterized protein yieL
167. YIFB_ECOLI Uncharacterized protein YifB
168. YJGZ_ECOLI Uncharacterized protein yjgZ
169. YJJK_ECOLI Uncharacterized ABC transporter ATP-binding protein YjjK
170. YJJU_ECOLI Uncharacterized protein yjjU
171. YKFF_ECOLI UPF0401 protein ykfF
172. YLBA_ECOLI Uncharacterized protein ylbA
173. YMFQ_ECOLI Uncharacterized protein YmfQ in lambdoid prophage e14 region
174. YNHG_ECOLI Probable L,D-transpeptidase YnhG
175. YPDC_ECOLI Uncharacterized HTH-type transcriptional regulator YpdC
176. YPJA_ECOLI Uncharacterized outer membrane protein YpjA
177. YQIG_ECOLI Putative outer membrane usher protein yqiG

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