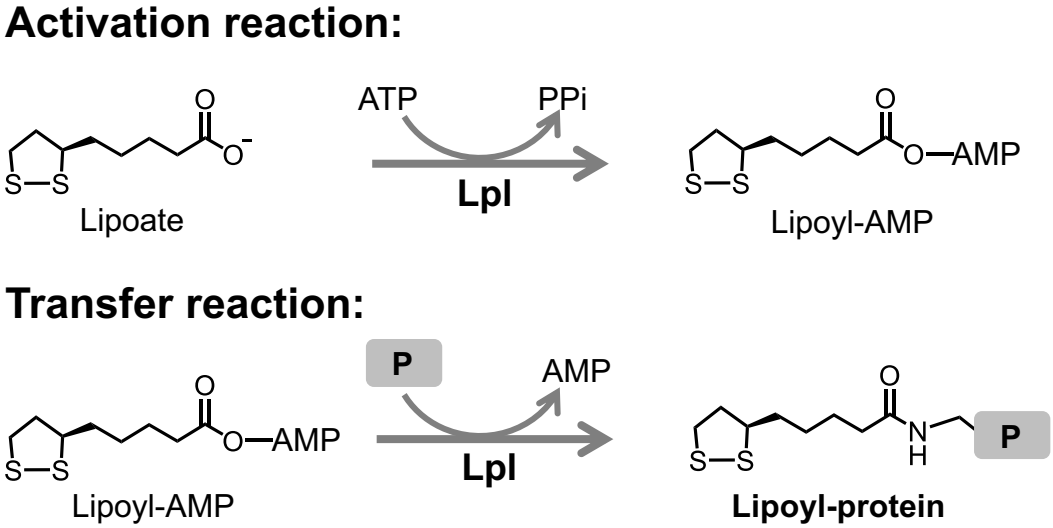


FIG S1. The two-step reaction catalyzed by lipoate-protein ligase (Lpl). Abbreviations: PPi, pyrophosphate; P, lipoate-dependent protein.

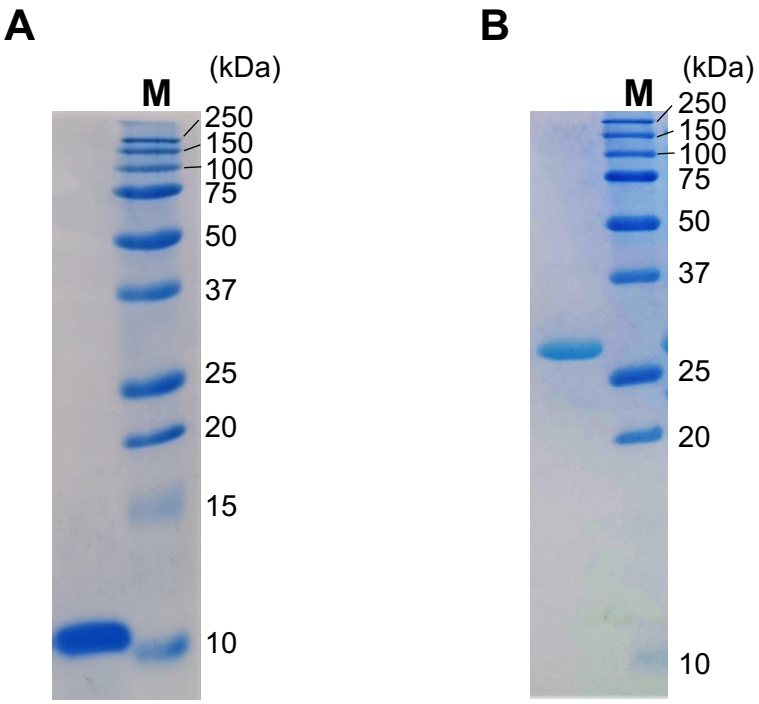


FIG S2. SDS-PAGE analysis of the purified TK1234 (A) and TK1908 (B) recombinant proteins. Four micrograms each of purified TK1234 and TK1908 recombinant proteins were applied. Gels were stained with Coomassie Brilliant Blue. M indicates molecular mass marker.

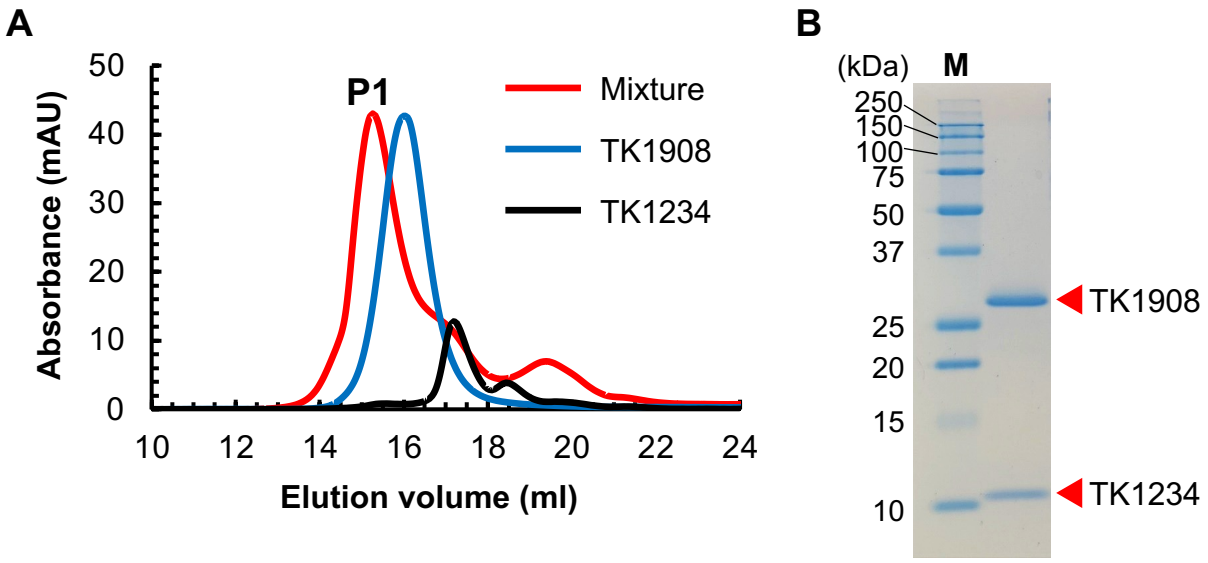


FIG S3. Association analysis of TK1234 and TK1908 proteins. (A) Chromatogram of gel filtration chromatography analyzing TK1234 protein (100 μM), TK1908 protein (100 μM), and their mixture. A Superdex 200 Increase 10/300 GL column was used. The mixture was composed of equimolar TK1908 protein and TK1234 protein (100 μM each). (B) SDS-PAGE analysis of a fraction corresponding to the peak P1 in the protein mixture solution (15.0 ml to 15.5 ml). M indicates the molecular mass marker.

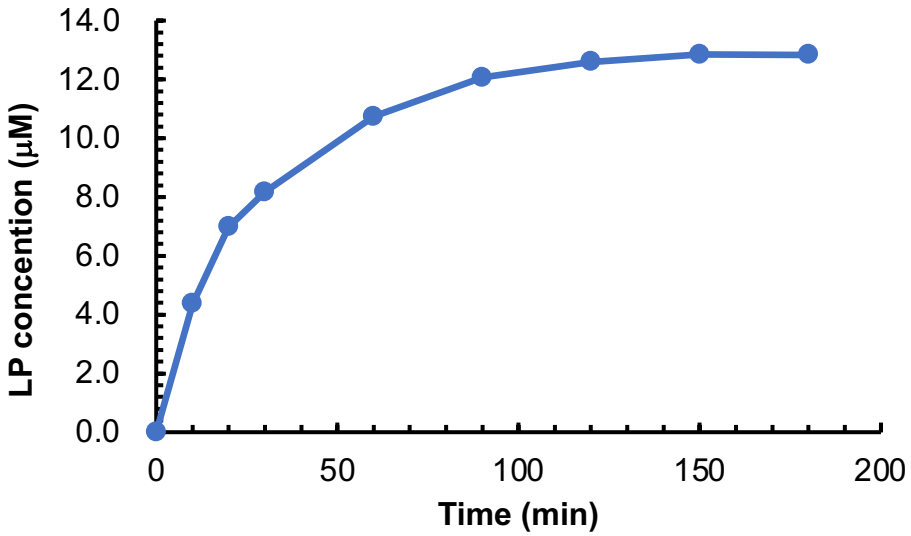


FIG S4. Progress curve of lipoyl-peptide (LP) formation catalyzed by single TK1908 protein. The reaction mixture was composed of 50 mM HEPES (pH 7.5 at 85^{o}C), 2 mM $MgCl_2$, 10 mM lipoate, 100 μM synthetic peptide, 5 mM ATP, and 0.7 μg/μl TK1908 protein.

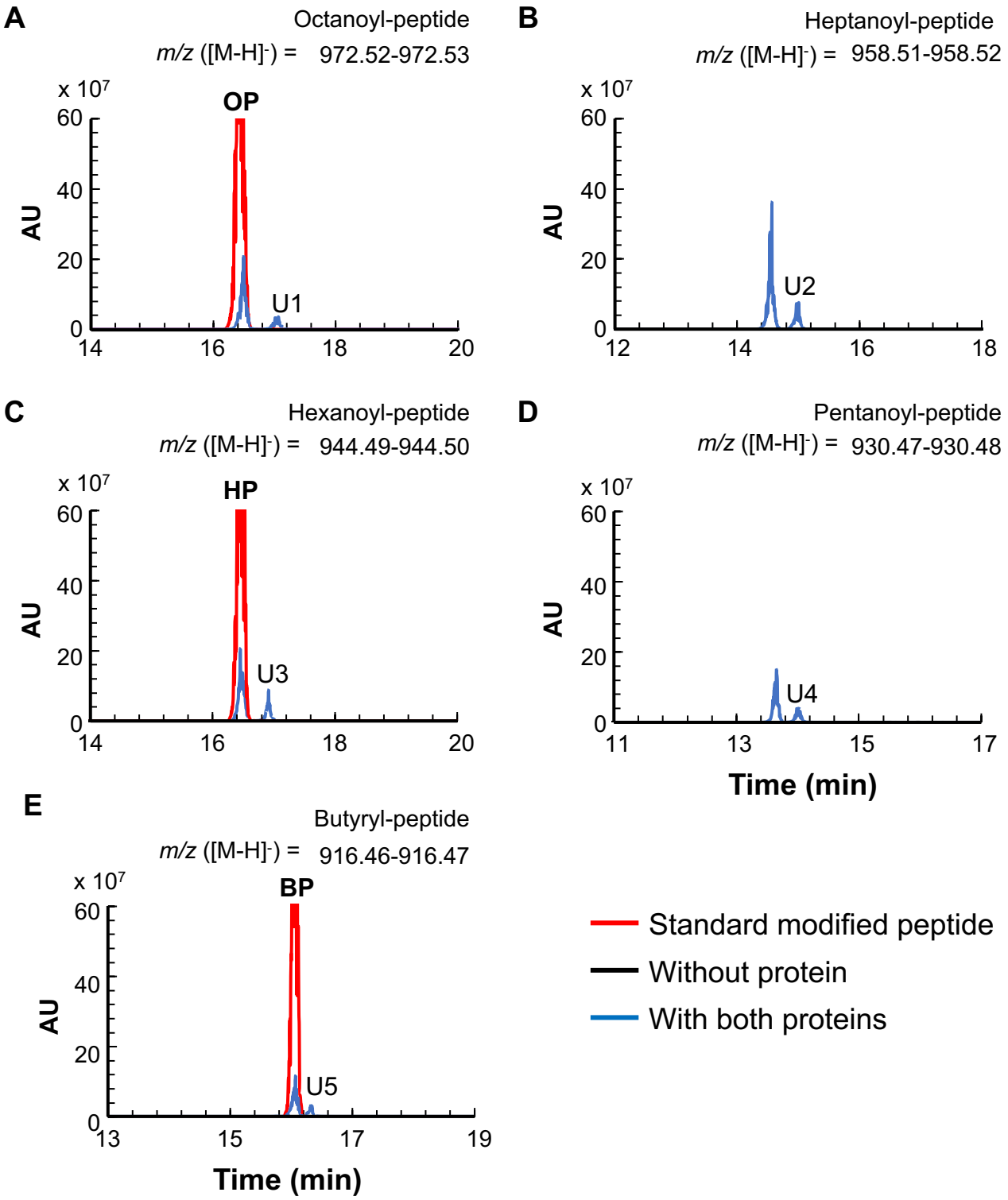


FIG S5. Identification of ligase reaction products by LC-MS analysis. The reactions were carried out with octanoate (A), heptanoate (B), hexanoate (C), pentanoate (D) and butyrate (E) as substrates and their reaction products were individually examined. Standard modified peptides were chemically synthesized. The chromatograms of the compounds whose exact masses corresponded to those of the modified peptides are shown. U1-U5 indicate the by-products with the same exact masses to those of modified peptides. Abbreviations: OP, standard octanoyl-peptide; HP, standard hexanoyl-peptide; BP, standard butyryl-peptide; AU, arbitrary units.

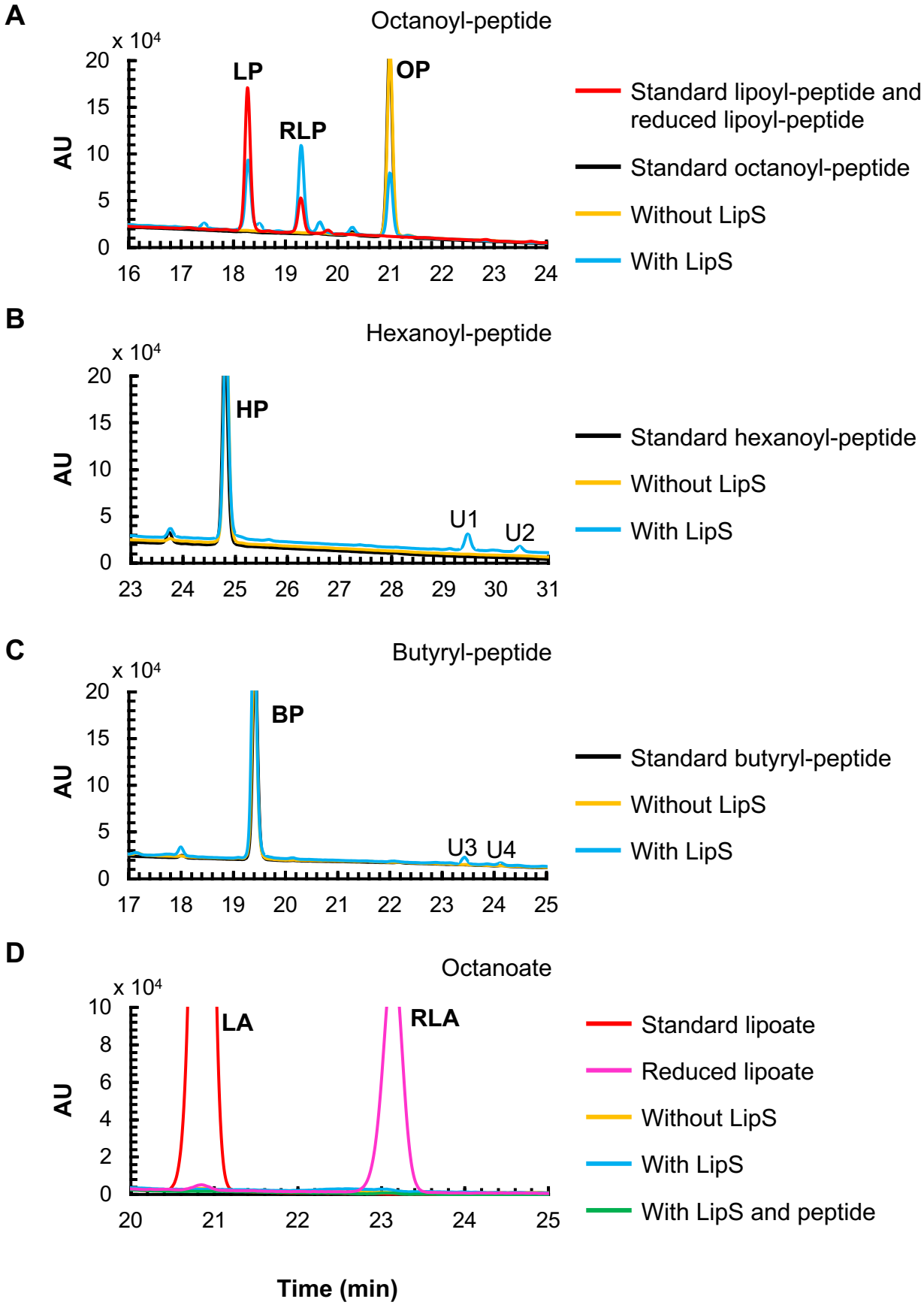


FIG S6. Lipoyl synthase activity measurement with HPLC. The reactions were carried out with octanoyl-peptide (A), hexanoyl-peptide (B), butyryl-peptide (C), and free octanoate (supplemented with or without peptide) (D) as substrates and their reaction products were individually examined. Standard lipoyl-peptide, standard octanoyl-peptide, standard hexanoyl-peptide, and standard butyryl-peptide were chemically synthesized. Abbreviations: LP, lipoyl-peptide; RLP, reduced lipoyl-peptide; OP, octanoyl-peptide; HP, hexanoyl-peptide; BP, butyryl-peptide; LA, lipoate; RLA, reduced lipoate; U1-U4, unidentified compounds; AU, arbitrary units.

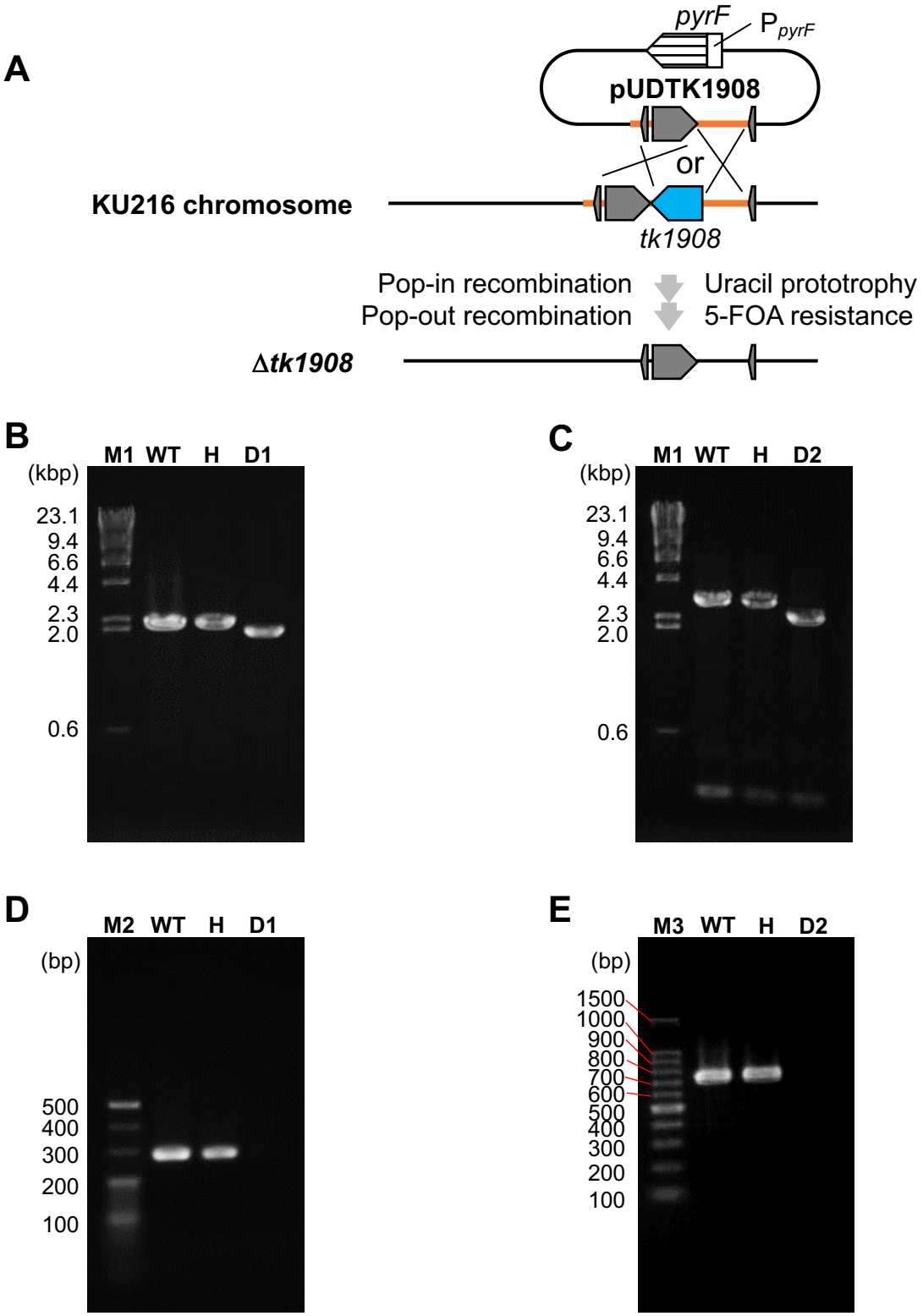


FIG S7. Construction of gene disruption mutants. (A) Schematic drawing for gene disruption in *T. kodakarensis*. As an example, the disruption of TK1908 is shown. *T. kodakarensis* KU216 (Δ*pyrF*) was used as the host strain. Pop-in/pop-out strategy using a *pyrF* gene as a selectable marker was applied. The gene disruption strain ΔTK1234 was constructed with the same method. (B-E) Genotypic analyses of the gene disruption strains. PCR analyses of the TK1234 and TK1908 gene disruption strains (ΔTK1234 and ΔTK1908) were carried out with primer sets that anneal outside of the TK1234- and TK1908-flanking regions for homologous recombination (B and C), respectively, and within the TK1234 and TK1908 genes (D and E), respectively. Abbreviations: M1, λ-Hind III marker (λ-phage DNA digested with HindIII); M2, 20 bp DNA Ladder (Dye Plus) (Takara Bio); M3, 100 bp DNA Ladder (Dye Plus) (Takara Bio); WT, *T. kodakarensis* wild-type KOD1; H, the host strain KU216; D1, ΔTK1234; D2, ΔTK1908.

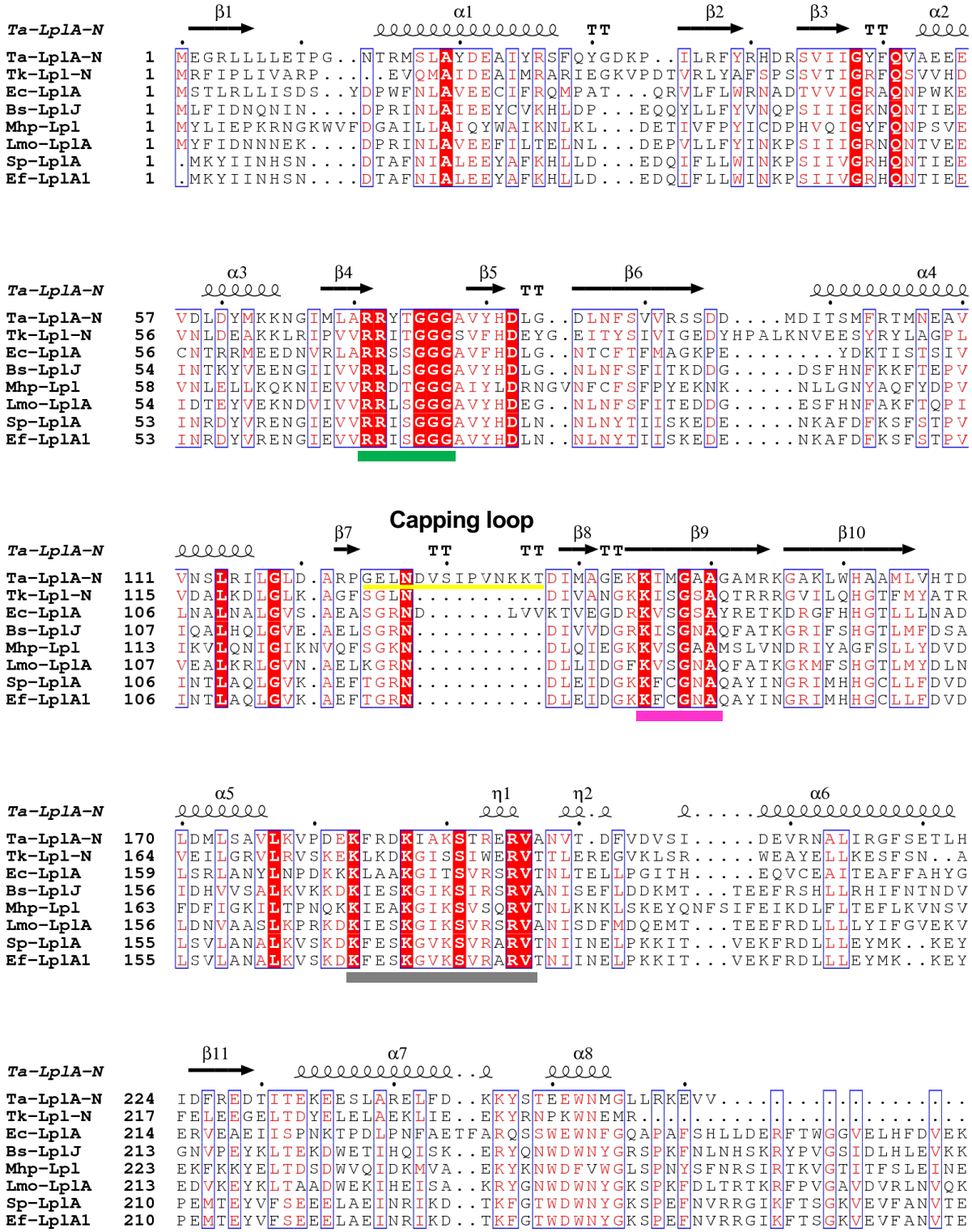


FIG S8. Amino acid sequence alignment of the large domains of Lpls. The sequences of *Tk*-Lpl-N (TK1908) and the large domains of *T. acidophilum* Lpl (*Ta*-LplA-N), *E. coli* Lpl (*Ec*-LplA), *B. subtilis* Lpl (*Bs*-LplJ), *M. hyopneumoniae* Lpl (*Mhp*-Lpl), *L. monocytogenes* Lpl (*Lmo*-LplA), *Streptococcus pneumoniae* Lpl (*Sp*-LplA), and *Enterococcus faecalis* Lpl (*Ef*-LplA1) were aligned. The secondary structure elements of *Ta*-LplA-N (PDB entry 2ARS) are shown above the sequence (squiggles, α-helices; arrows, β-strands; TT, β-turns; η, 3/10-helices). The RRX(T/S)GGG, KX_2GXA, and $KX_3KX_3SX_3RV$ motifs conserved among Lpl members are indicated by green, magenta and gray bars, respectively. The capping loop motif of *Ta*-LplA-N (residues 124-137) is underlined in yellow. Similar residues in the columns are shown by red letters, and identical residues are shown by white letters on a red background. The columns that display high similarity (global score higher than 0.7) are framed in blue. The dots above the alignment indicate every 10th residue of the top sequence.

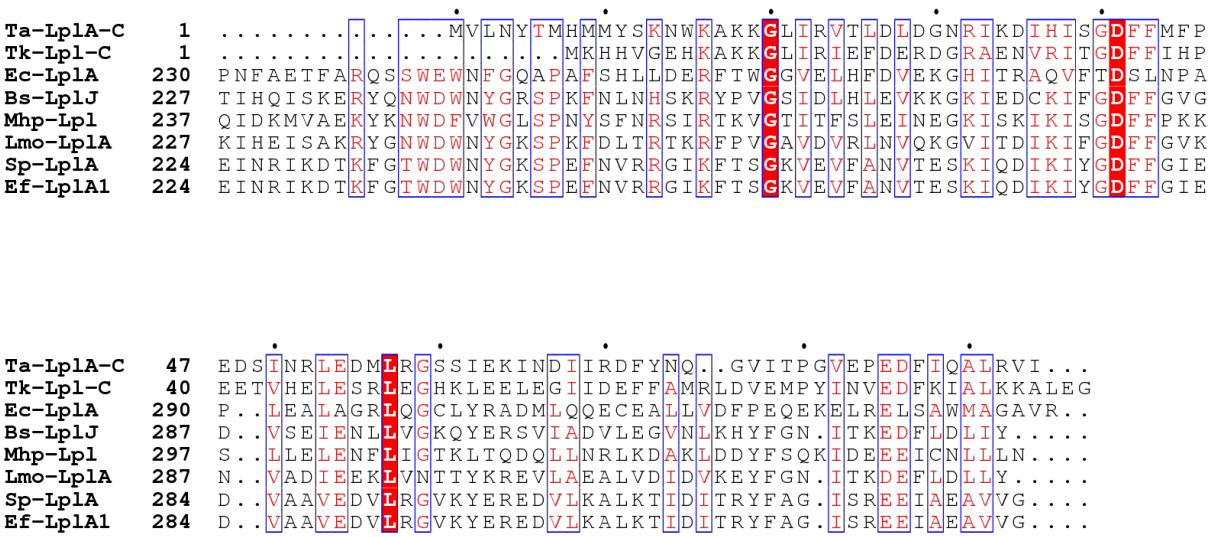


FIG S9. Amino acid sequence alignment of the small domains of Lpls. The sequences of *Tk*-Lpl-C and the small domains of *T. acidophilum* Lpl (*Ta*-LplA-C), *E. coli* Lpl (*Ec*-LplA), *B. subtilis* Lpl (*Bs*-LplJ), *M. hyopneumoniae* Lpl (*Mhp*-Lpl), *L. monocytogenes* Lpl (*Lmo*-LplA), *S. pneumoniae* Lpl (*Sp*-LplA), and *E. faecalis* Lpl (*Ef*-LplA1) were aligned. Residues are colored and indicated as described in Fig. S8.

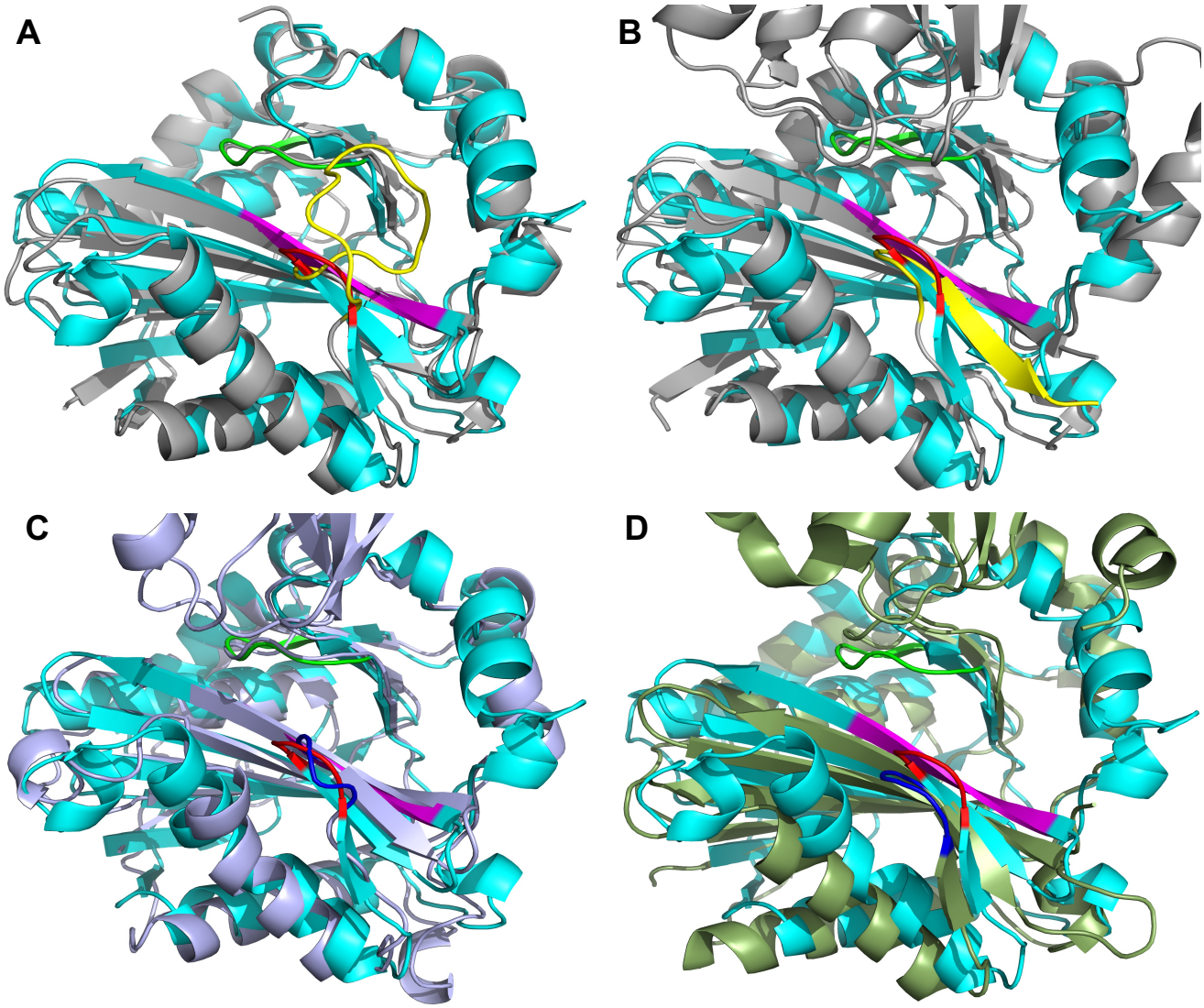


FIG S10. Structural superimpositions of *Tk*-Lpl-N with Lpls from other species. Structural superimpositions of *Tk*-Lpl-N (cyan) with the large domain of *T. acidophilum* Lpl (*Ta*-LplA-N, PDB entry 2ARS, gray) (A), *T. acidophilum* Lpl (*Ta*-LplA, PDB entry 3R07, gray) (B), *M. hyopneumoniae* Lpl (*Mhp*-Lpl, PDB entry 6JOM, light blue) (C), and *E. coli* Lpl (*Ec*-LplA, PDB entry 1X2G, dark green) (D). Only the large domains are shown here. The conserved motifs RRX(T/S)GGG and KX_2GXA in the active site of *Tk*-Lpl-N structure are indicated in green and magenta, respectively. The capping loop motif and the corresponding regions of *Tk*-Lpl-N (red), *Ta*-LplA-N (yellow), *Ta*-LplA (yellow), *Mhp*-Lpl (blue), and *Ec*-LplA (blue) are colored.

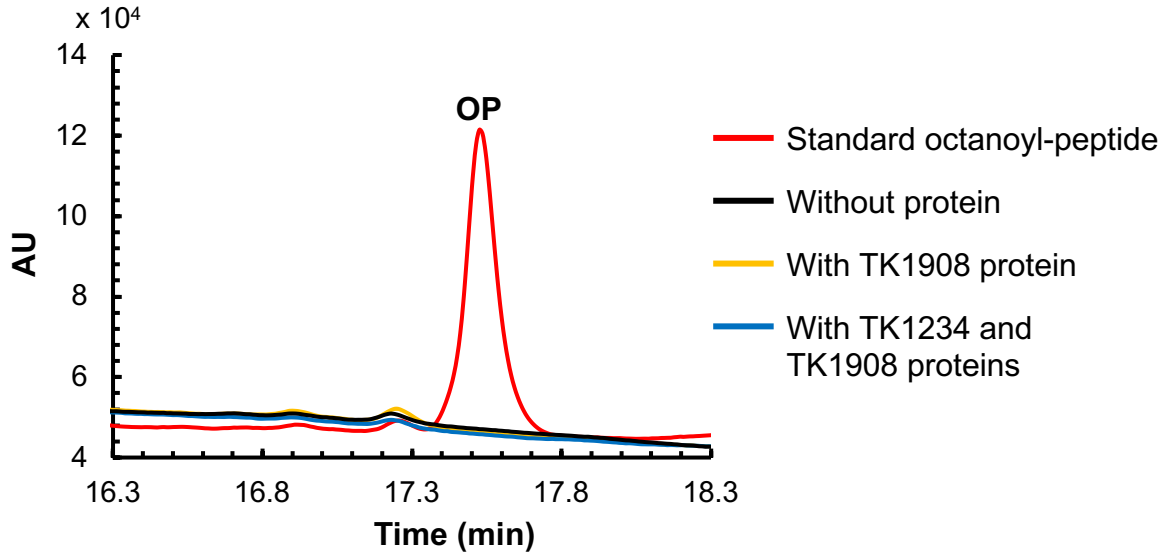


FIG S11. Octanoyl transferase activity measurement with HPLC. The octanoyl transferase activity was determined based on a previous study (1). The reaction mixture (50 μl) was composed of 50 mM HEPES (pH 7.5 at 85ºC), 100 mM KCl, 5 mM DTT, 100 μM synthetic peptide, 100 μM octanoyl-coenzyme A (CoA), 5 μg TK1908 protein (and 5 μg TK1234 protein). The reaction mixture was incubated at 85ºC for 1 h and analyzed with HPLC. Abbreviations: OP, octanoyl-peptide; AU, arbitrary units.

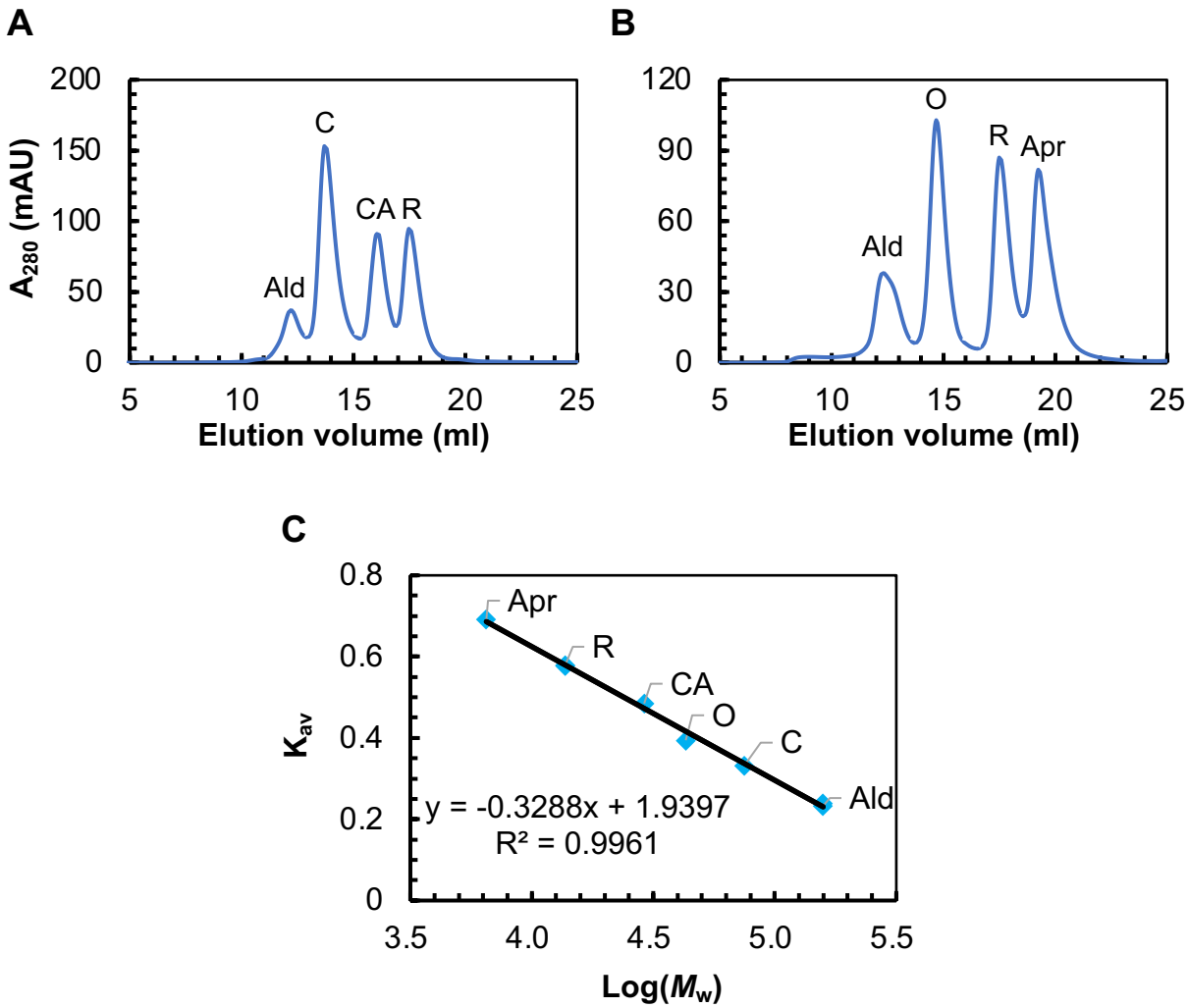
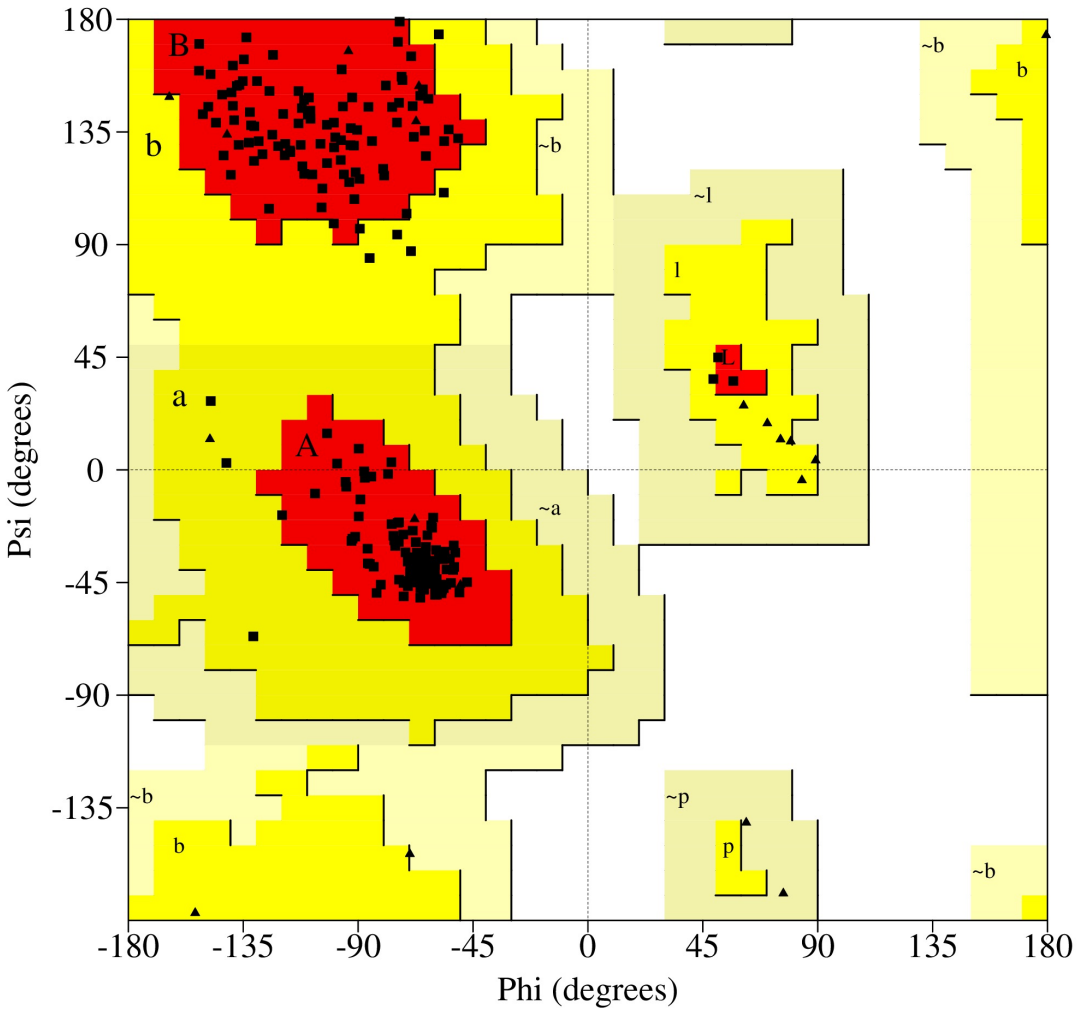


FIG S12. Standard curve of proteins used in the determination of molecular mass with gel filtration chromatography. (A) and (B) Chromatograms of the standard proteins used for calibration curve plotting. The absorbance was monitored at 280 nm. The standard proteins are aprotinin (Apr, 6.5 kDa), ribonuclease A (R, 13.7 kDa), carbonic anhydrase (CA, 29 kDa), ovalbumin (O, 43 kDa), conalbumin (C, 75 kDa) and aldolase (Ald, 158 kDa). (C) Calibration curve. The distribution coefficient (K_{av}), which is calculated from the measured elution volume, against the logarithmic molecular mass Log(M_w) of each protein was plotted.



Plot statistics

Residues in most favoured regions [A,B,L]	211	95.9%
Residues in additional allowed regions [a,b,l,p]	9	4.1%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	220	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	19	
Number of proline residues	8	
Total number of residues	249	

FIG S13. Ramachandran plot of *Tk*-Lpl-N structure model. The Ramachandran plot obtained for the *Tk*-Lpl-N structure model quality assessment showed that 95.9% of the residues was in the most favored regions (red area), 4.1% was under the allowed regions (yellow and light-yellow areas), suggesting a good quality of the structure model. The model of *Tk*-Lpl-N was validated by PROCHECK module of UCLA-DOE LAB-SAVES v6.0.

Reference:

1. Nesbitt NM, Baleanu-Gogonea C, Cicchillo RM, Goodson K, Iwig DF, Broadwater JA, Haas JA, Fox BG, Booker SJ. 2005. Expression, purification, and physical characterization of *Escherichia coli* lipoyl (octanoyl) transferase. Protein Expr Purif 39:269-282.