Supplementary Data

Structural basis for the substrate recognition and catalysis of peptidyl-tRNA hydrolase

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Supplementary Materials and Methods

Docking simulation

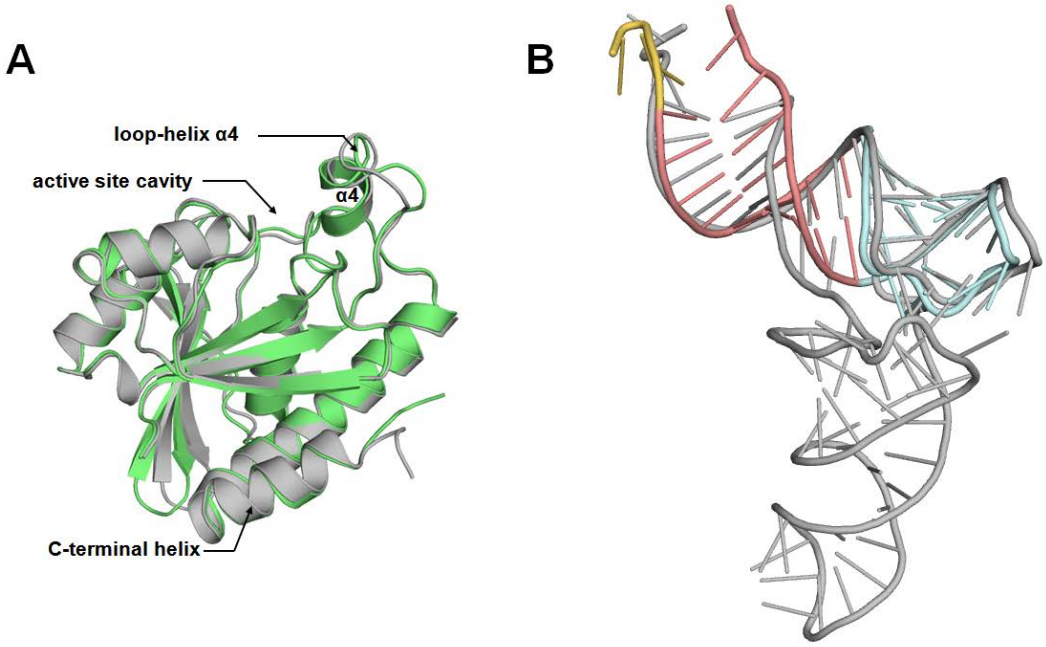
Docking simulations of tripeptidyl-adenosines to Pth were performed using AutoDock Vina (53) with default parameters. Gasteiger charges and non-polar hydrogen atoms were added using AutoDockTools (55). PDBQT molecular structure files were also prepared by AutoDockTools. All of the rotatable bonds of tripeptidyl-adenosines were allowed to rotate freely. The search area for docking was set to a 12 × 24 × 16 Å rectangular box, which encompassed the entire active site cavity, including Phe66.

Supplementary Results and Discussion

Structural comparison with other protein:tRNA complexes

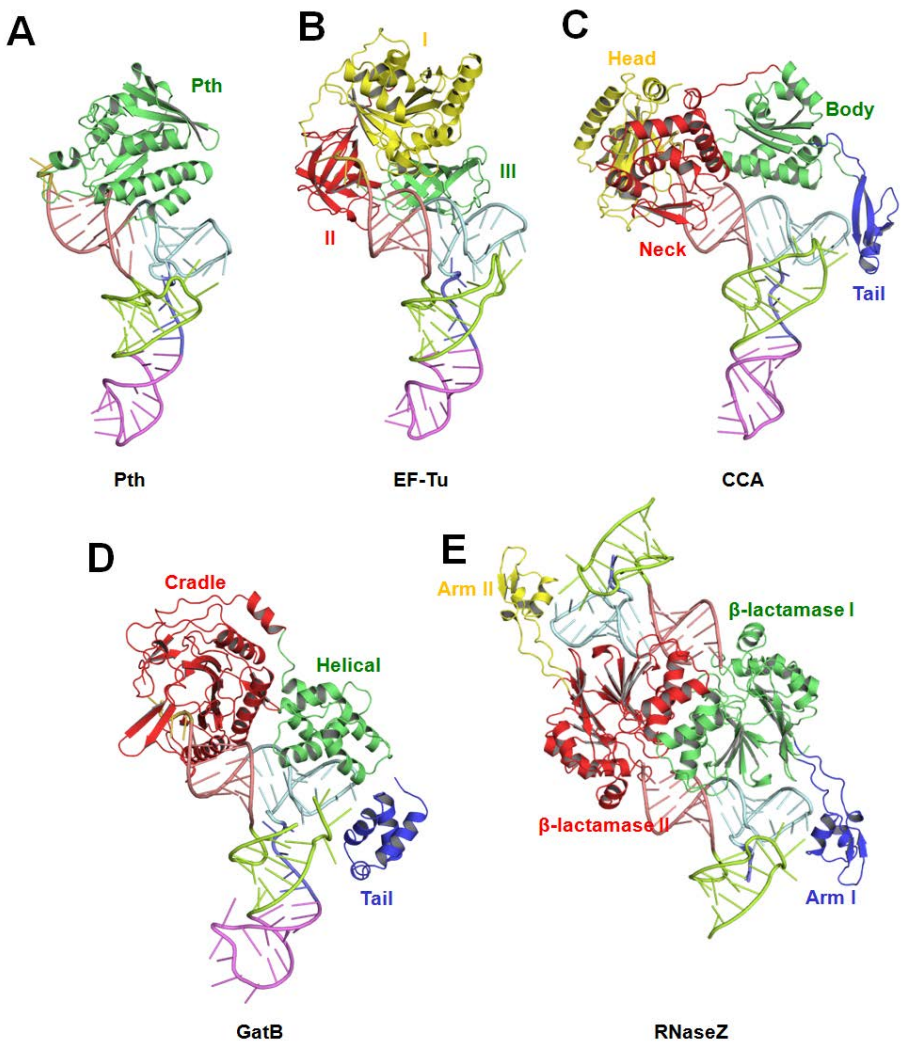
We compared the structure of the Pth:CCA-acceptor-TΨC domain complex with those of the other protein:tRNA complexes determined so far, in which the proteins recognize the acceptor and TΨC stem regions on the TΨC stem side (**Supplementary Figure S4**). The structural comparison revealed that Pth recognizes the acceptor stem and the TΨC stem of the substrate by its single domain, whereas the other proteins recognize them by multi-domain or multi-subunit systems. The details are as follows: **Supplementary Figure S4A** shows the Pth:tRNA^{Phe} complex model (to clarify the overall configuration of the tRNA recognition mode of Pth, yeast tRNA^{Phe} (40) is superposed onto the equivalent region of the Pth:CCA-acceptor-TΨC domain complex). As mentioned above, Pth recognizes the acceptor stem and the TΨC stem by a single, globular domain. **Supplementary Figure S4B** shows the EF-Tu:Phe-tRNA^{Phe} complex (42). EF-Tu, which mediates the entry of the aminoacyl-tRNA into the A site of the ribosome (56), recognizes the acceptor stem by domains I, II and III, and recognizes the TΨC stem by domain III (42). **Supplementary Figure S4C** shows a class I CCA-adding enzyme:tRNA^{Phe} complex (43). The CCA-adding enzyme is responsible for the addition of CCA onto the 3'-terminus of immature tRNAs, using CTP and ATP as substrates (57). This protein recognizes the acceptor stem by the neck and body domains, and identifies the TΨC arm by the body and tail domains (43,44). **Supplementary Figure S4D** shows the GatB:tRNA^{Asn} complex (45). GatB is a subunit of the bacterial GatCAB amidotransferase, which produces Gln-tRNA^{Gln} and Asn-tRNA^{Asn} from Glu-tRNA^{Gln} and Asp-tRNA^{Asn}, respectively (58). GatB recognizes the acceptor stem by its cradle domain, and the TΨC arm by its helical and tail domains (45,46). The structure of GatDE, an archaeal Glu-tRNA^{Gln} amidotransferase, has also been solved with tRNA (59).

This enzyme recognizes the substrate in a manner similar to that of GatCAB, and therefore the figure is not included. **Supplementary Figure S4E** shows the short form of the RNase Z:anticodon arm-truncated $tRNA^{Thr}$ complex (47). RNase Z catalyzes the endonucleolytic removal of the 3' extension of the tRNA precursors after the discriminator base (60). The enzyme is a homodimer consisting of two identical subunits, composed of a core zinc-dependent β-lactamase domain and a protruding flexible arm domain, and it binds to its substrates with a 2:2 stoichiometry. The acceptor stem of each substrate molecule is recognized by the β-lactamase domain of both subunits, and the TΨC arm is recognized by the arm domain of one subunit (47). As described above, Pth has a unique tRNA recognition feature.



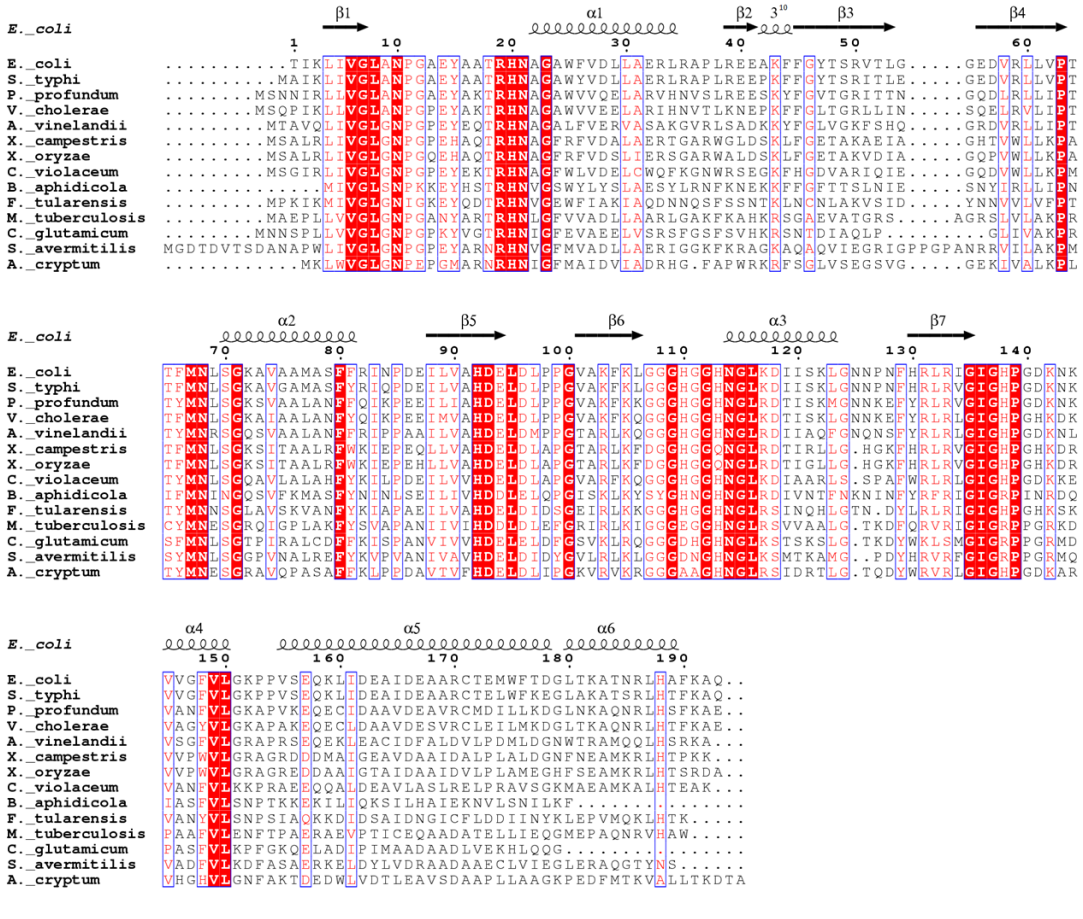
Supplementary Figure S1. Structural comparisons of Pth and the CCA-acceptor-TΨC domain.

(**A**) Superposition of the substrate-free *E. coli* Pth (gray, PDB accession code 2PTH) (25) and Pth from the Pth:CCA-acceptor-TΨC domain complex (green). (**B**) Superposition of free yeast $tRNA^{Phe}$ (gray, PDB accession code 6TNA) (40) and the CCA-acceptor-TΨC domain (same color-coding as in **Figure 2**).

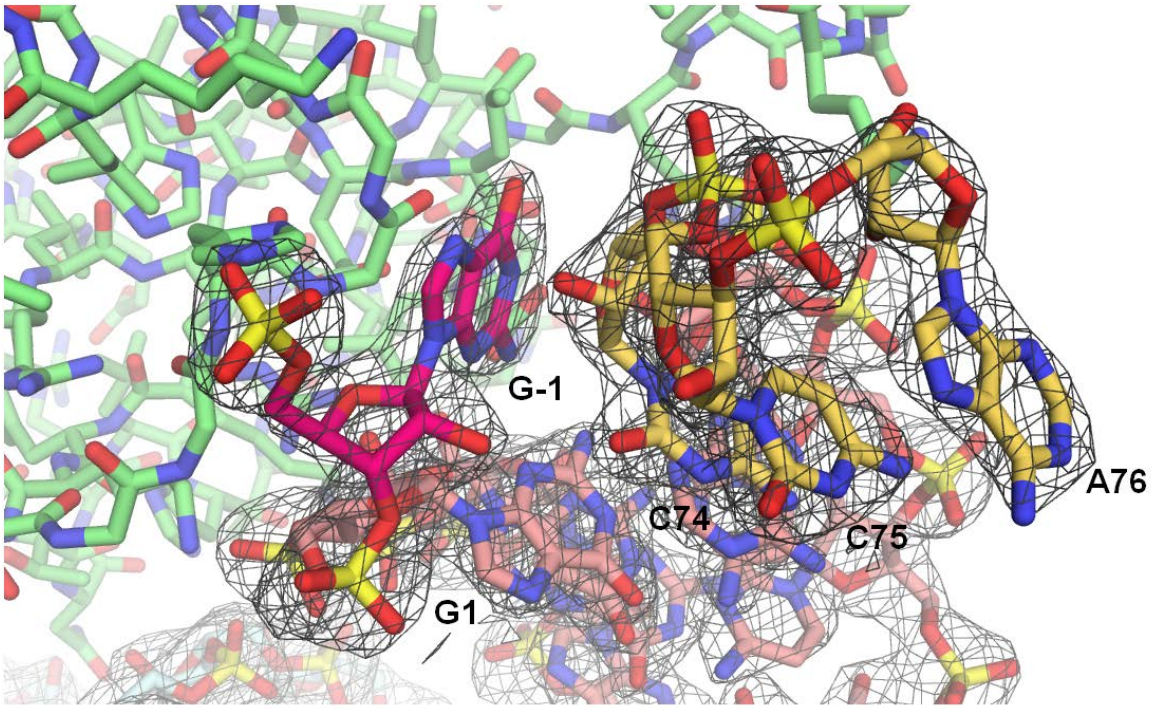


Supplementary Figure S2. Structure comparison with other protein:tRNA complexes. All proteins are depicted by ribbon diagrams. (**A**) Pth:tRNA^{Phe} complex model. To clarify the overall configuration of the tRNA recognition mode of Pth, yeast tRNA^{Phe} (PDB accession code 6TNA) (40) was superposed onto the equivalent region of the Pth:CCA-acceptor-TΨC domain complex. Pth is colored green. In all panels (**A**-**E**), the color-coding of the tRNA is the same as that in **Figure 1**. (**B**) EF-Tu:Phe-tRNA^{Phe} complex (PDB accession code 1TTT) (42). Domains I, II and III of EF-Tu are colored yellow, red and green, respectively. (**C**) Class I CCA-adding enzyme:tRNA^{Phe} complex (PDB accession code 1SZ1) (43). The head, neck, body and tail domains of the CCA-adding enzyme are

colored yellow, red, green and blue, respectively. (**D**) GatB:tRNA^{Asn} complex (PDB accession code 3KFU) (45). The cradle, helical and tail domains of GatB are colored red, green and blue, respectively. (**E**) Short form RNase Z:anticodon arm-truncated tRNA^{Thr} complex (PDB accession code 2FK6) (47). RNase Z is a homodimeric enzyme, and it binds to its substrates with a 2:2 stoichiometry. The β-lactamase and flexible arm domains of one subunit and the same domains of the other subunit are colored green, blue, red and yellow, respectively.

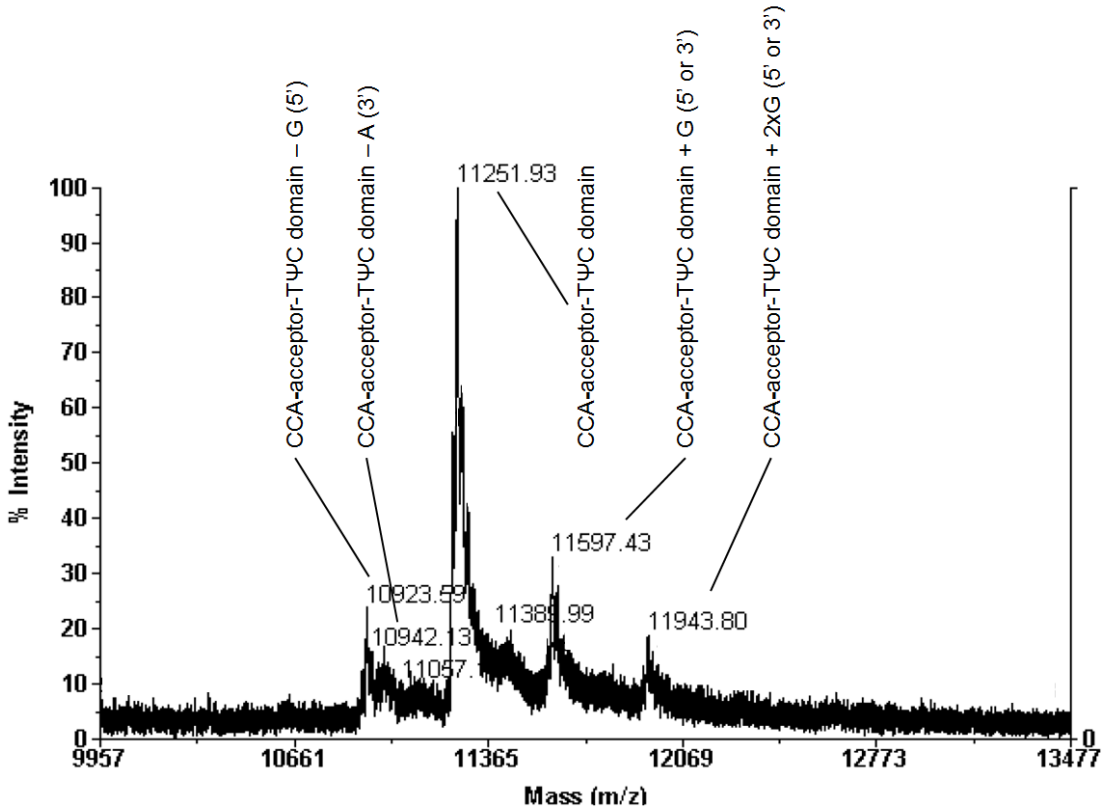


Supplementary Figure S3. Amino acid sequence alignment of prokaryotic Pths. Conserved amino acid residues are shown by red characters. Strictly conserved amino acid residues are indicated by a red background. The secondary structure of *E. coli* Pth is shown above the sequences. The figure was produced by ESPript 2.2 (http://espript.ibcp.fr/ESPript/ESPript/).



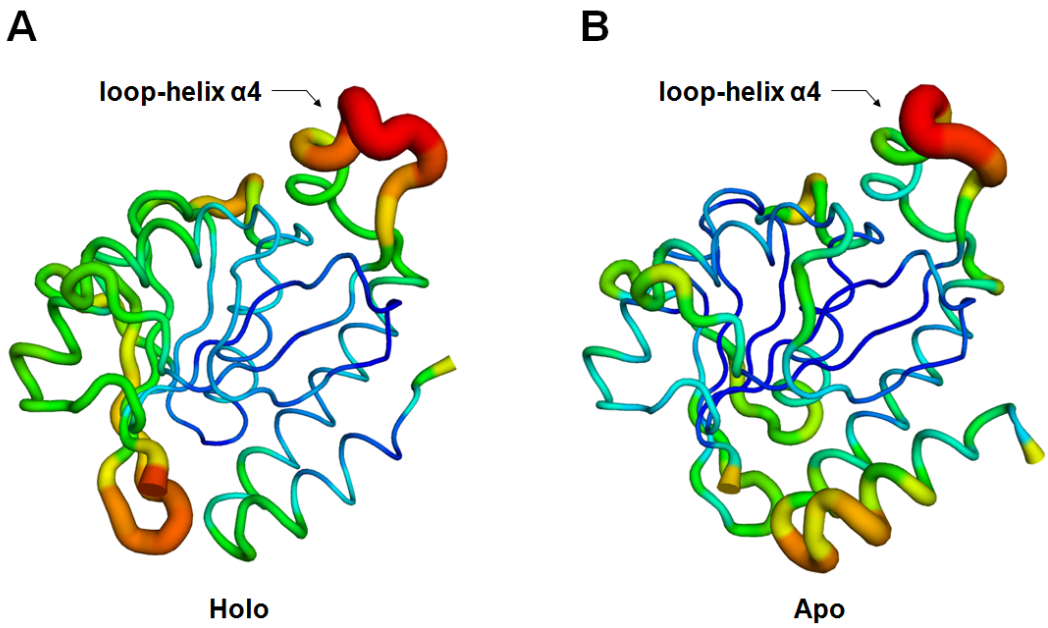
Supplementary Figure S4. Structure around the extra G-1.

The structure around the extra G-1 is represented by a stick model. The extra G-1 is colored magenta, the acceptor stem is pink, the TΨC arm is cyan, the CCA terminus is yellow, and Pth is green. The 2*Fo* – *Fc* electron density map surrounding the CCA-acceptor-TΨC domain is also shown (contoured at sigma = 1.5). The extra G-1 is sandwiched between Pth and the 3'-CCA terminus, and supports the flexible 3'-CCA terminus.



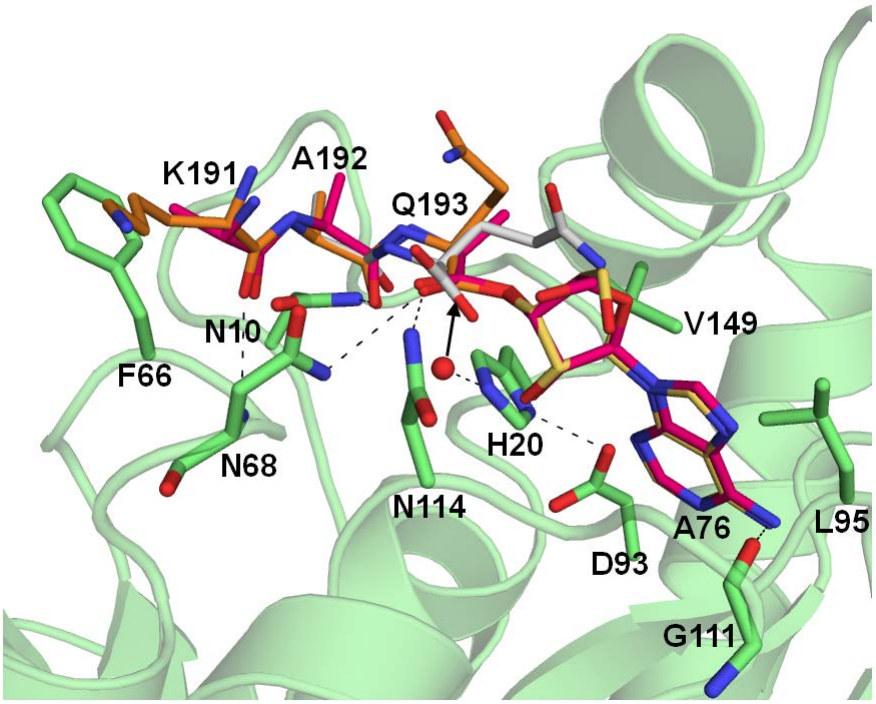
Supplementary Figure S5. Mass spectrum analysis of the transcription products.

The transcription products were analyzed by a Voyager DE Pro MALDI-TOF mass spectrometer (AB SCIEX), using 3-hydroxypicolinic acid as a matrix. In the figure, the features of the products that correspond to each peak are indicated. The minor peaks corresponding to m/z = 11057 and 11389 were not assigned.



Supplementary Figure S6. Temperature factors of Pth.

(**A**) Temperature factors of Pth, from the Pth:CCA-acceptor-TΨC domain complex (Holo). (**B**) Temperature factors of the substrate-free *E. coli* Pth (Apo, PDB accession code 2PTH) (25). The relative temperature factor is indicated by a color gradient from blue (low) to red (high).



Supplementary Figure S7. Docking simulation of the tripeptidyl-adenosine.
As in **Figure 5**, Pth (green), A76 (yellow) and the peptide moiety (orange) of the Pth:peptidyl-tRNA complex model are represented by stick models on a transparent ribbon model of Pth. On this model, the best result from the docking simulation of the tri-alanyl-adenosine to Pth is represented by a red stick model. The K191-A192-Q193 tripeptide from the crystallographically neighboring molecule in the substrate-free Pth structure (25) is also represented by a grey stick model. Note that the region of K191 in the Pth:peptidyl-tRNA complex model and that of the substrate-free Pth structure overlap completely; therefore, the region of K191 in the substrate-free Pth structure is not visible.

At first, we performed the docking simulation using the K191-A192-Q193 tripeptidyl-adenosine as a search molecule. However, we could not obtain a solution in which the adenine ring fits into the active site cavity. It seemed likely that the presence of the side chains caused difficulty in the calculation. Therefore, we used tri-alanyl-adenosine as a search molecule in the simulation, and obtained the solution as depicted in this figure.

Supplementary References

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