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Supporting Information

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Supporting Information

for

Site-Specific Protein Cross-Linking with Genetically Incorporated 3,4-Dihydroxy-L-phenylalanine

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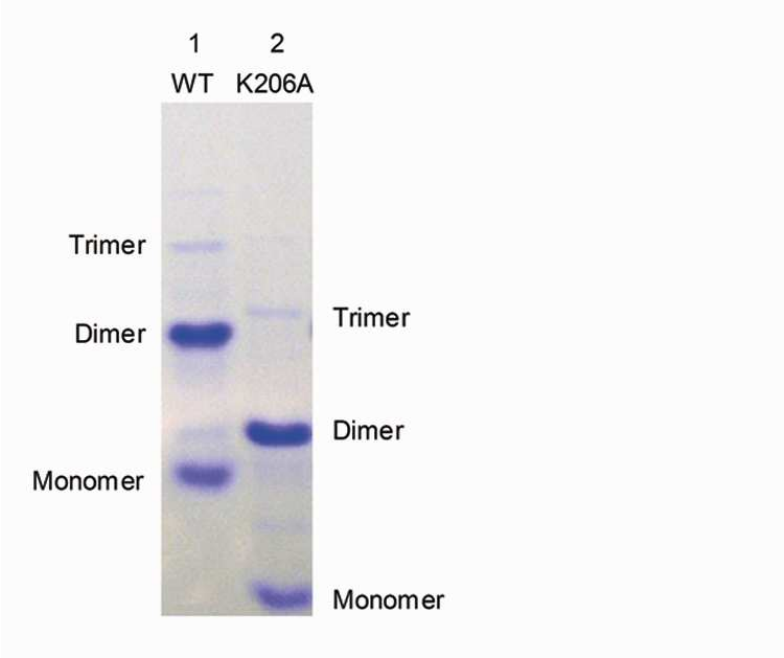

Figure S1: Native PAGE of purified $SrtA_{\Delta N59}$ and $SrtA_{\Delta N59}$K206A proteins. Wild type (lane 1) and K206A mutant (lane 2) $SrtA_{\Delta N59}$ proteins were resolved by non-denaturing PAGE and visualized by Coomassie Brilliant Blue staining. Mobilities of the wild type and the mtant proteins depend on their indivisual isoelectric points as well as molecular weights, and are consistent with the data for other $SrtA_{\Delta N59}$ mutants (Zhu, et al, *Biochemistry* **2008**, *47*, 1667). In each lane, 4μL of 5 mg/mL protein was loaded.

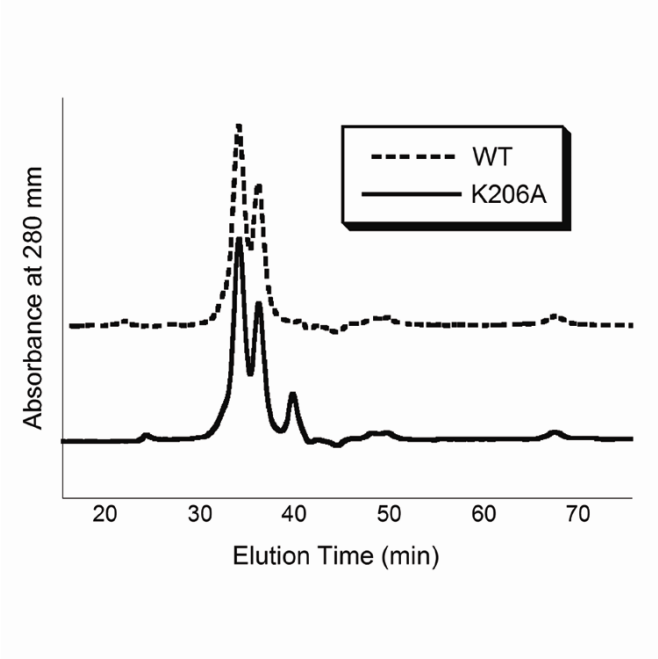

Figure S2: Gel filtration chromatography of purified $SrtA_{\Delta N59}$ and $SrtA_{\Delta N59}$K206A proteins. Wild type (dotted line) and K206A mutant (solid line) $SrtA_{\Delta N59}$ proteins were applied to a gel filtration column and eluted with PBS (100 mM NaH_2PO_4, 150 mM NaCl, pH 6.8) at 0.75 mL/min. at 4°C. For each run, 100μL of 5 mg/mL protein was injected. The elution pattern was monitored by measuring the absorbance at 280 nm.

Experimental Section

Vector constructions. Coding sequence of $SrtA_{\Delta N59}$-His6 was previously cloned into the expression vector pET28b (Novagen, La Jolla, CA) to generate the plasmid pET28-SrtA59. The following mutant vectors carrying either amber stop codon (TAG) or codon for alanine (GCG) at the designated residues were generated using the primer pairs listed below (Invitrogen, Carlsbad, CA):

pET28-SrtA59K137TAG: 5'-CGATCCATGGGCCAAGCTAAACCTCAAATTCC-3' and 5'-CAACTTTAAAGTACACCATACTACCTTTCTAGGCTGCTTTAAG-3'.

pET28-SrtA59K206TAG: 5'-GTAGCTACAGAAGTCTAGCTCGAGCACCACCACC-3' and 5'-GGTGGTGGTGCTCGAGCTAGACTTCTGTAGCTAC-3'.

pET28-SrtA59K206A: 5'-GTAGCTACAGAAGTCGCGCTCGAGCACCACCACC-3' and 5'-GGTGGTGGTGCTCGAGCGCGACTTCTGTAGCTAC-3'.

The fragment of the gene which contains the mutation was amplified by polymerase chain reaction (PCR) using pET28-SrtA59 plasmid as the template. The PCR product was double-digested with NcoI and DraI, and then ligated with pET28-SrtA59 also di-

gested with NcoI and DraI to afford pET28-SrtA59K137TAG. Vectors pET28-SrtA59K206TAG and pET28-SrtA59K206A were generated using QuickChange® Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) according to the manufacturer's manual, using pET28-SrtA59 and pET28-SrtA59K206TAG as templates, respectively. All vector sequences were confirmed by DNA sequenceing (The University of Texas at Austin ICMB DNA Core Facility). The vector pAC-DHPheRS-6TRN which carries one copy of mutant *Methanococcus jannaschii* Tyr-tRNA synthetase and six copies of mutant *M. jannaschii* $tRNA^{Tyr}$ was a gift from Dr. Peter G. Schulz (The Scripps Research Institute, La Jolla, CA).

Site-Specific Incorporation of 3,4-dihydroxy-L-phenylalanine (L-DOPA) into $SrtA_{\Delta N59}$. In a typical procedure of incorporation, *Escherichia coli* BL21 (Novagen, La Jolla, CA) was first chemically transformed with the plasmid pET28-SrtA59K137TAG or pET28-SrtA59K206TAG and plated on Luria-Bertani (LB) agar plates supplemented with kanamycin (50 μg/mL). Resulting single colonies were inoculated into LB broth (50 μg/mL kanamycin) and made electro-competent. These competent cells were transformed with the second plasmid pAC-DHPheRS-6TRN by electroporation and plated on LB agar plates containing kanamycin (50 μg/mL) and tetracycline (12.5 μg/mL). Single colonies were subcultured in LB broth supplemented with kanamycin (50 μg/mL), tetracyclin (12.5 μg/mL) and glucose (1 mM), and grown overnight at 37°C. The cells in the small starting cultures were collected, resuspended in sterile deionized water, and inoculated into glucose minimal media supplemented with kanamycin (50 μg/mL) and tetracycline (12.5 μg/mL). Cultures were grown at 37°C for 14 hours until OD_{600} reached approximately 0.7. Tetracycline concentration was increased (18.75 μg/mL) and 3,4-dihydroxy-L-phenylalanine (L-DOPA, 1 mM, Acros Organics, NJ) was added. Cultures were further incubated at 30°C for 40 min before the expression of DOPA-containing $SrtA_{\Delta N59}$ proteins was induced with isopropyl β-D-1-thiogalactopyranoside (IPTG, 1 mM, Gold Bioechnology, Inc., St. Louis, MO). Cultures were grown for additional 5 h at 30°C and the cells were harvested and frozen at -80°C.

Cross-linking of DOPA-containing $SrtA_{\Delta N59}$ Dimer and Protein purification. The frozen cells obtained above were thawed on ice and resuspended in lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0). Sodium periodate (1 mM, Acros Organics, NJ) was added and the mixtures were incubated on ice for 1 h. Lysozyme

(1 mg/mL) was added and the samples were incubated on ice for additional 30 min. Samples were then sonicated and centrifuged at 15,000 rpm for 40 min at 4°C. The cell lysates were collected and applied to Ni-NTA agarose beads (Qiagen, Madison, WI) pre-equilibrated with lysis buffer. The beads were washed extensively with wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0). The His_6-tagged proteins were then eluted with elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 500 mM imidazole, pH 8.0). To optimize the experimental procedures, the concentration of sodium periodate was varied between 1 to 2.5 mM depending on the experiment. The incubation time after adding the sodium periodate and lysozyme was also varied between 20-70 min and 30-60 min, respectively. The wash buffer was modified to contain one or all of the following: glycerol (10%), Tween-20 (0.1%), β-mercaptoethanol (20 mM), higher concentrations of NaCl (2 M) and imidazole (30 mM). Elution buffer containing a lower concentration of imidazole (250 mM) was also used in some experiments.

Polyacrylamide gel electrophotresis and Western blotting. DOPA-containing $SrtA_{\Delta N59}$ proteins were resolved by 15% SDS-polyacrylamide gel electrophoresis (PAGE) under denaturing condition and visualized by Coomassie Brilliant Blue staining. For Western blot analyses, proteins were transferred to a nitrocellulose membrane in Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (Bio-Rad, Hercules, CA) using Tris-glycine transfer buffer (48 mM Tris-base, 3.9 mM glycine, 0.037% SDS, 20% methanol, pH 8.3). The membrane was probed with anti-His C-terminal antibody conjugated with alkaline phosphatase (Invitrogen, Carlsbad, CA). Phospha GLO AP substrate (KPL, Inc., Baltimore, MD) was applied to visualize the proteins and the signals were detected by exposing the membrane to BioMax light film (Eastman Kodak Co., Rochester, NJ).

Redox-cycling staining of DOPA-containing proteins. A similar membrane carrying $SrtA_{\Delta N59}$-containing L-DOPA was obtained as described in the previous section. DOPA-containing proteins were visualized by staining the membrane with nitroblue tetrazolium (NBT). The membrane was incubated in the NBT staining solution (2 M sodium glycinate, 0.24 mM NBT, pH 10.0) in darkness for 3 h.

Purification of Wild Type and K206A Mutant $SrtA_{\Delta N59}$. *Escherichia coli* BL21 was transformed with the plasmid pET28-SrtA59 or pET28-SrtA59K206A and plated on LB agar plates containing kanamycin (50 mg/mL). Resulting single colonies were in-

oculated into LB broth supplemented with kanamycin (50 µg/mL) and grown at 37°C until OD_{600} reached 0.7. Over-expression of wild-type and mutant $SrtA_{\Delta N59}$ was induced with 1 mM IPTG and the cultures were grown for additional 5 h at 37°C. The cells were harvested and frozen at -80°C. The cells were then resuspended in wash buffer and incubated on ice for 1 h in the presence of lysozyme (1 mg/mL). The samples were sonicated and centrifuged at 15 000 rpm for 40 min at 4°C. The cell lysates were collected and applied to Ni-NTA agarose beads pre-equilibrated with wash buffer. Unbound proteins were washed off with wash buffer and the His6-tagged proteins were eluted with elution buffer. Final protein concentrations were calculated by measuring the absorbance at 280 nm using NanoDrop™ spectrophotometer (Thermo Scientific, Wilmington, DE).

Nondenaturing PAGE. Appropriate dilutions were made for the purified wild type and K206A mutant $SrtA_{\Delta N59}$ proteins to a total protein concentration of 5 mg/mL. The samples were then incubated at 4°C for at least 24 h. Approximately 20 µg of total protein was loaded in each lane and resolved by native-PAGE using 14% Tris-glycine polyacrylamide gel (Invitrogen, Carlsbad, CA). The gels were visualized by Coomassie Brilliant Blue staining.

Gel filtration chromatography. The same protein samples prepared in the previous section was also analyzed using Superdex™ 200 10/300 GL column (GE Healthcare, Sweden) equipped with BioLogic DuoFlow system (Bio-Rad, Hercules, CA). The column was pre-equilibrated with phosphate-buffered saline (PBS, 100 mM NaH_2PO_4, 150 mM NaCl, pH 6.8) overnight prior to the sample application. For each run, 100 µL of purified protein (5 mg/mL) was injected and eluted with PBS at 0.75 mL/min at 4°C. Protein fractions were detected by monitoring the absorbance at 280 nm.