Electronic Supplementary Information

Transpeptidation-Directed Intramolecular Bipartite Tetracysteine Display for Sortase Activity Assay

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EXPERIMENTAL SECTION

Materials. The TC-FlAsH II in-cell tetracysteine tag detection kit was purchased from Invitrogen (U.S.A.). Recombinant human Hdm2 protein was obtained from R&D Systems (Minneapolis, U.S.A.). The T4 DNA Ligase was purchased from TaKaRa (Dalian, China). The curcumin was obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). The CytoSelect™ Cell Adhesion Assay kit (Fibrinogen-Coated, Colorimetric Format) was purchased from Cell Biolabs, Inc. (CA, U.S.A.). The peptide Dabcyl-QALPETGEE-Edans and the peptides (Table S1) were synthesized by SciLight Biotechnology LLC (Beijing, China) with purity greater than 95%. The *S. aureus* strain was obtained from American Type Culture Collection (Manassas, VA, U.S.A.). The Sortase A pentamutant (eSrtA) in pET29 (i.e.,

pET29-SrtA pentamutant) was a gift from David Liu (Addgene plasmid # 75144). All experiments were performed using deionized, filtered water from a MilliQ system (Millipore, Billerica, MA, U.S.A.).

Table S1. Peptide sequences and their binding affinity to FlAsH-EDT$_2$

Name	Peptide sequences (N- to C-terminal)	K_d^{app} (μM) [a]
aPP-N	Ac-CCPSQPTYPGLPATGG-NH_2 [b]	>1000
aPP-C	GGVEDLIRFYDNLQQYLNVCC-NH_2 [b]	356.47 ± 28.61
CC-aPP- CC	A_c-CCPSQPTYPGLPATGGVEDLIR**F**YDNLQQ**Y**LNVCC-NH_2 [c]	5.01 ± 0.31
CC-aPP^P-CC	A_c-CCPSQPTYPGLPATGGVEDLIRPYDNLQQPLNVCC-NH_2	71.93 ± 1.24

[a] The K_d^{app} was determined by adding increasing amount of peptides to bipartite tetracysteine display buffer containing 0.1 μM FlAsH-EDT$_2$ and 0.05 mM BAL.

[b] The sequence derived from avian pancreatic polypeptide is underlined.

[c] The residues that are mutated to proline to generate CC-aPP^P-CC are indicated in bold.

Protein Expression and Purification. The pET29-SrtA pentamutant was transformed into bacteria BL21(DE3) for protein expression. The transformed cells were cultivated in LB-media containing kanamycin (50 μg/mL), and the protein expression was induced by adding 0.5 mM isopropyl β-D-thiogalactoside (IPTG). The cells were grown at 30℃ for 4 h. Bacteria were harvested by centrifugation and stored at -80 ℃ until use. For protein purification, the cells were suspended in 20 mL of lysis buffer (50 mM Tris·HCl, pH 7.5, 150 mM NaCl, 10 mM imidazole) followed by sonication and clarification. The supernatant was then applied to a Ni-NTA (Qiagen) column. The column was washed with wash buffer I (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 20 mM imidazole) and wash buffer II (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 40 mM imidazole) to remove the unspecific binding proteins. The SrtA^{5M} protein

was subsequently eluted with an elution buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 200 mM imidazole). The eluted proteins were further purified on a Superose 6 10/300 GL gel filtration column (GE Healthcare). The isolated protein was analyzed on a 15% SDS-PAGE and the purity was estimated to be > 95 %. The $SrtA^{5M}$ proteins were concentrated to 3.7 mg/mL in SrtA reaction buffer (50 mM Tris·HCl, pH 7.5, 150 mM NaCl, 5 mM $CaCl_2$, 1 mM DTT) and stored at -80 ℃.

MALDI-TOF MS Analysis. All peptides were dissolved in DMSO to make a stock solution (4 mM). The molecular weights of all peptides were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). For the aPP-N/aPP-C mixture and CC-aPP-CC, a certain amount of peptides were added into 20 μL of SrtA reaction buffer and incubated at 37 ℃ for 2 h. For the SrtA-catalyzed transpeptidation reaction, 10 μM aPP-N, 40 μM aPP-C, and 0.1 μg of $SrtA^{5M}$ were added into 20 μL of SrtA reaction buffer and incubated at 37 ℃ for 2 h. The reaction mixtures were 10-fold diluted with ultrapure water before being subjected to MS analysis. All the samples were analyzed with a Voyager DE STR MALDI-TOF mass spectrometer (Applied Biosystems, U.S.A.).

$SrtA^{5M}$-Mediated Transpeptidation Reaction and Fluorescence Measurement. For the transpeptidation reaction, 10 μM aPP-N, 10 μM aPP-C, and 0.1 μg of $SrtA^{5M}$ were added into 20 μL of SrtA reaction buffer and incubated at 37 ℃ for 2 h. The reaction mixture was subsequently added to 80 μL of bipartite tetracysteine display buffer (100 mM Tris·HCl, pH 7.4, 75 mM NaCl, 1 mM EDTA, 1 mM DTT), followed by the addition of 0.4 μM FlAsH-EDT_2 and 0.05 mM BAL. The mixture was incubated at room temperature for 30 min before fluorescence measurement. The fluorescence signal was measured using a FSP920 fluorescence spectrometer (Edinburgh Instruments, U.K.) with an

excitation wavelength of 510 nm. To verify the specificity of aPP-N and aPP-C to $SrtA^{5M}$, the aPP-N/aPP-C mixture was incubated with 0.1 μg of $SrtA^{5M}$, 0.1 μg of Hdm2, and 0.1 μg of T4 DNA ligase, respectively. The reaction mixture was subsequently subjected to fluorescence measurement and fluorescence imaging. To determine the limit of detection (LOD), the reaction was performed with a constant concentration of aPP-N(10 μM) and aPP-C(10 μM), and variable concentrations of $SrtA^{5M}$. The fluorescence signal of aPP-N/aPP-C mixture without any treatment was measured as well. The change in fluorescence intensity is calculated according to eq 1:

$$\Delta F = F_{SrtA} - F_{negative} \quad (1)$$

where ΔF represents the increase in fluorescence intensity, $F_{negative}$ is the fluorescence intensity in the absence of $SrtA^{5M}$, and F_{SrtA} is the fluorescence intensity in the presence of $SrtA^{5M}$. The calculated ΔF is plotted against the concentration of $SrtA^{5M}$ to determine the LOD.

For the fluorogenic peptide cleavage assay, 10 μM Dabcyl-QALPETGEE-Edans and 10 μM aPP-C were mixed with varying concentrations of $SrtA^{5M}$ in 100 μL of SrtA reaction buffer at 37 °C for 2 h. Meanwhile, the fluorescence signal of Dabcyl-QALPETGEE-Edans/aPP-C mixture without $SrtA^{5M}$ treatment was measured. The fluorescence signals were measured by a FSP920 Fluorescence spectrometer with an excitation wavelength of 340 nm.

Determination of apparent equilibrium dissociation constants (K_d^{app}). The **apparent** equilibrium dissociation constants (K_d^{app}) of biarsenical−peptide complexes were determined by monitoring the increase of fluorescence as a function of peptide concentration. The titrations were performed in bipartite tetracysteine display buffer, and the K_d^{app} value was obtained as previously described. [1, 2] Specifically, increasing concentrations of peptides from 0.5 μM to 256 μM were added to 100 μL of bipartite tetracysteine display buffer containing 0.1 μM FlAsH-EDT_2 and 0.05 mM BAL. The mixture was incubated at room temperature for 30 min. The emission at 526 nm was used to calculate K_d^{app} value.

Inhibition of SrtA^{5M} Activity by Curcumin. The curcumin was dissolved in DMSO. For the inactivation of SrtA^{5M}, variable-concentration curcumin (0, 2, 3, 4, 10, 20, 30, 40, 200, and 300 μg/mL) were added to 20 μL of SrtA reaction buffer containing 0.1 μg of SrtA^{5M} and incubated at room temperature for 10 min. The activity of curcumin-treated SrtA^{5M} was determined by adding 10 μM substrate peptides to the reaction buffer and incubated at 37 ℃ for 2 h. The reaction mixture was diluted with 80 μL of bipartite tetracysteine display buffer followed by staining with biarsenical dye. The inhibitory rate was calculated using eq 2:

$$\text{Inhibitory rate (\%)} = (1 - F_i/F_0) \times 100\% \quad (2)$$

where F_0 is the net fluorescence intensity without curcumin treatment, F_i is the net fluorescence intensity that obtained following curcumin treatment. The IC_{50} value was determined using sigmoidal dose–response by Origin software. Each data point was obtained from five independent measurements.

Inhibition of SrtA Activity in *S. aureus* Cells. The *S. aureus* cells were cultured in brain heart infusion (BHI) broth and harvested when cells reached an optical density of ~0.6 at 600 nm. The *S. aureus* cells were resuspended in SrtA reaction buffer and ultrasonicated for 30 s. Subsequently, 0.1% Tween-20 was added to the lysate and incubated at room temperature for 20 min. The cell debris was removed by centrifugation and the supernatant was incubated with 10 μM aPP-N/40 μM aPP-C at 37 ℃ for 2 h. The aPP-N/aPP-C mixture without any treatment was used as a control. For the inhibition of endogenous SrtA, variable-concentration curcumin (0, 1, 3, 5 μg/mL) was added to the supernatant before incubation with aPP-N and aPP-C. The reaction mixtures were subsequently stained with FlAsH-EDT_2 and the fluorescence signals were measured by a FSP920 spectrometer.

Growth of *S. aureus* Cells in the Presence of Curcumin. The *S. aureus* cells were diluted 1:1000 into fresh BHI medium containing variable-concentration curcumin (0, 5, and 10 μg/mL). The *S. aureus* cells were grown at 37 ℃

with shaking and the samples were taken at indicated time points. Absorbance at 600 nm was measured using an UV-Vis spectrophotometry (METASH).

Fibrinogen-Binding Assay. Approximately 150 μL of *S. aureus* cell suspension was added to fibrinogen-coated 48-well plate (Cell Biolabs) and incubated at 37 ℃ for 90 min. After washing with 250 μL of PBS for four times, the plates were stained with 200 μL of cell stain solution for 10 min at room temperature. The wells were washed with 500 μL of deionized water for four times followed by the addition of 200 μL of extraction solution. The absorbance at 560 nm was subsequently measured using a MULTISKAN GO microplate spectrophotometer (Thermo Scientific).

Statistical Analysis. The statistical significances of two groups were calculated using the 2-tailed Student's t-test. Differences were considered statistically significant when $P < 0.01$.

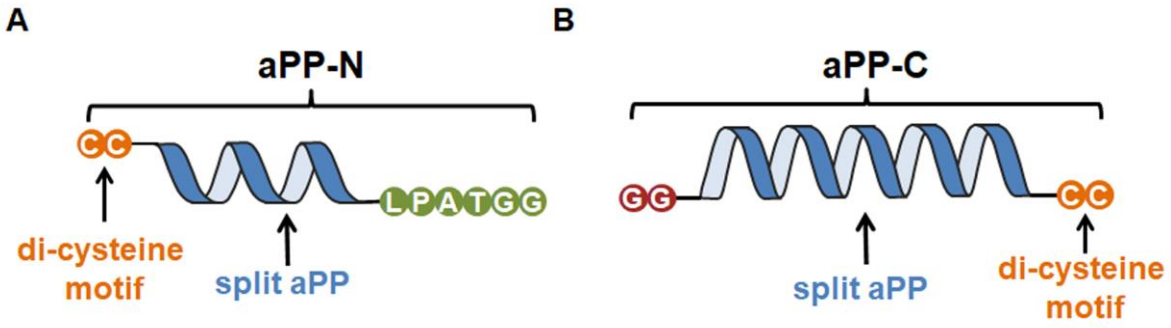

Fig. S1 Domain organization of aPP-N (A) and aPP-C (B). The di-cysteine motif (deep orange) and the sequence required for transpeptidation reaction (olive green for aPP-N and brown for aPP-C) was linked by two aPP fragments (blue).

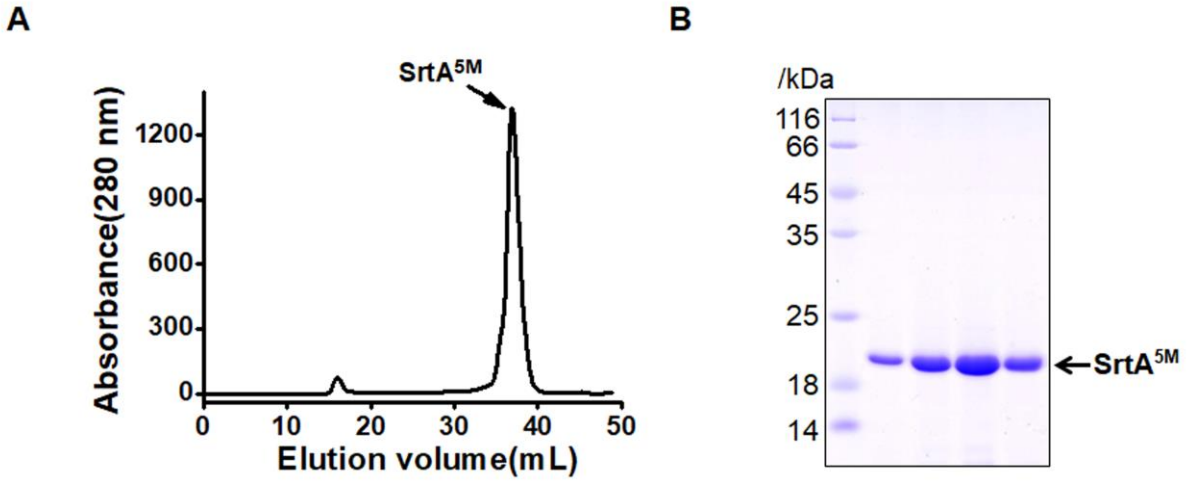

Fig. S2 Purification and characterization of SrtA^{5M}. (A) The gel filtration chromatograph of SrtA^{5M}. (B) Analysis of eluted SrtA^{5M} on a 15% SDS-PAGE followed by Coomassie staining.

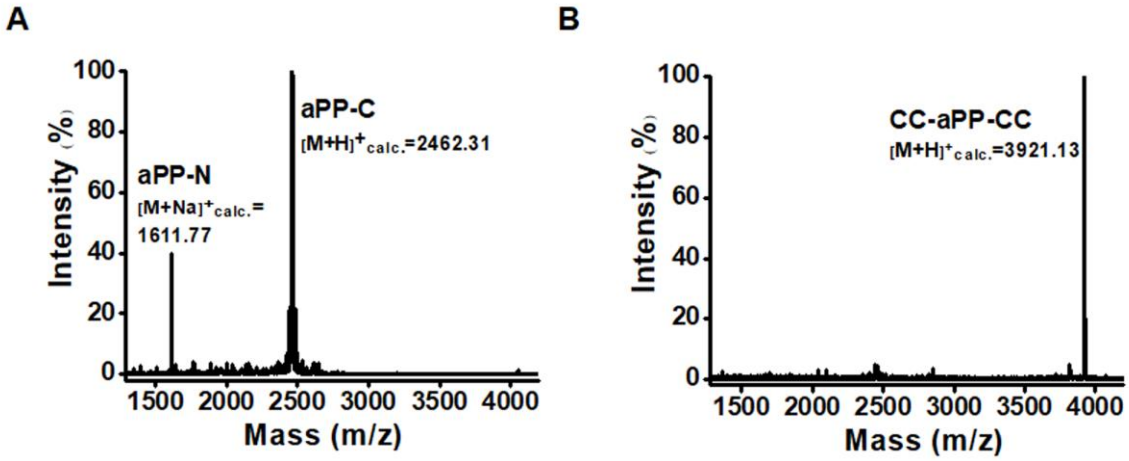

Fig. S3 MALDI-TOF spectrum of aPP-N/aPP-C mixture (A) and CC-aPP-CC (B).

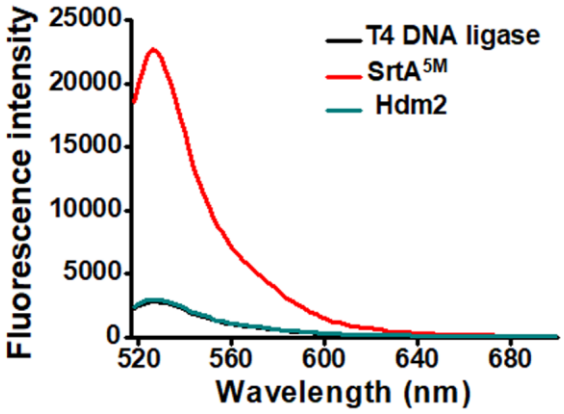

Fig. S4 Fluorescence spectra of FlAsH-EDT$_2$ upon binding to aPP-N/aPP-C mixture that was pre-incubated with T4 DNA Ligase (black), SrtA^{5M} (red), and Hdm2 (dark cyan), respectively.

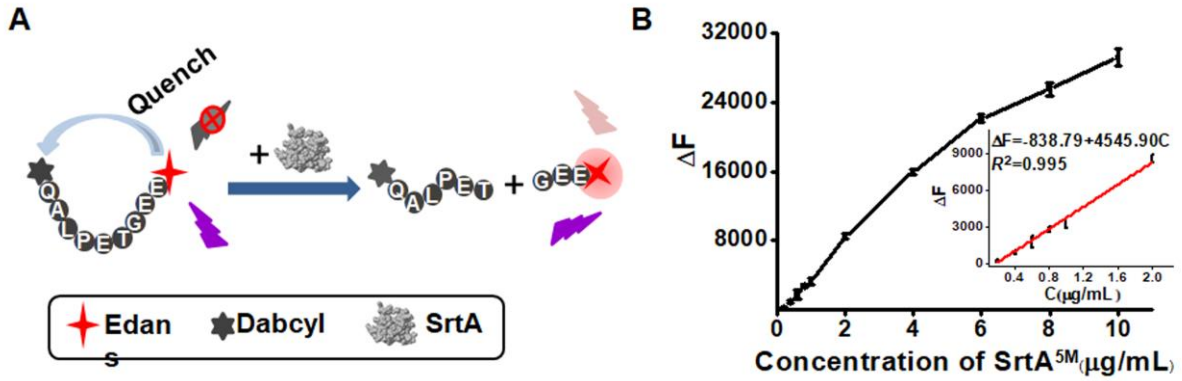

Fig. S5 Measurement of SrtA^{5M} by the fluorogenic peptide cleavage assay. (A) Schematic illustration of fluorogenic peptide cleavage assay. (B) Variance of the fluorescence increment at 490 nm (ΔF) *vs* the concentration of SrtA^{5M}. The inset shows the linear relationship between the fluorescence increment (ΔF) and the SrtA^{5M} concentration in the range from 0.2 to 2 μg/mL. Error bars show the standard deviation of five independent experiments.

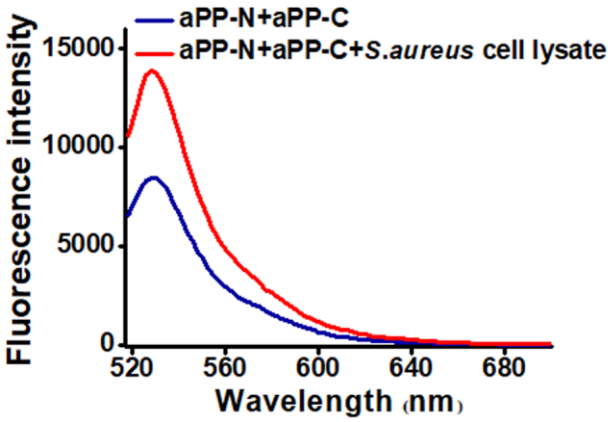

Fig. S6 Fluorescence spectra of FlAsH-EDT$_2$ upon binding to aPP-N/aPP-C mixture that was pre-incubated with (red) and without (blue) *S. aureus* cell lysate.

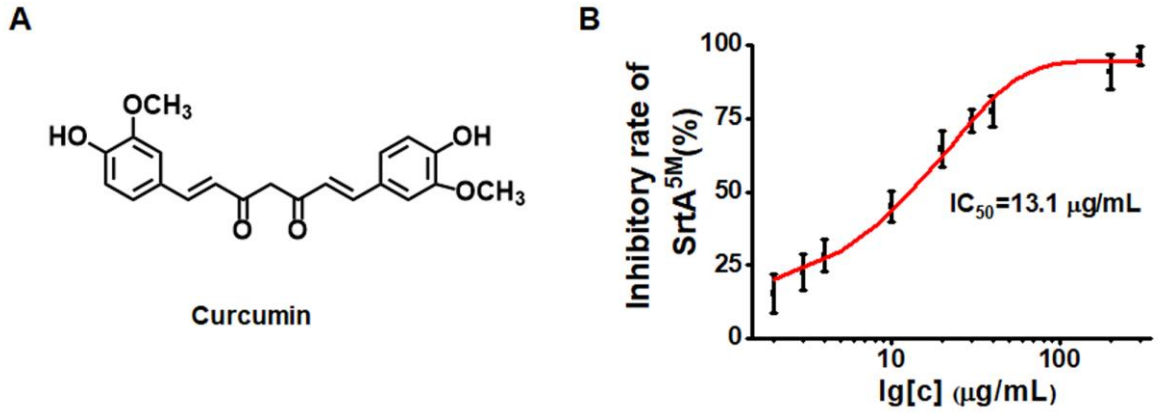

Fig. S7 Characterization of curcumin-mediated inhibition of SrtA activity by transpeptidation-directed intramolecular bipartite tetracysteine display. (A) Chemical structure of curcumin. (B) Inhibition of $SrtA^{5M}$ activity by curcumin in a dose-dependent manner. The results are shown as the mean values ± SD of five replicates.

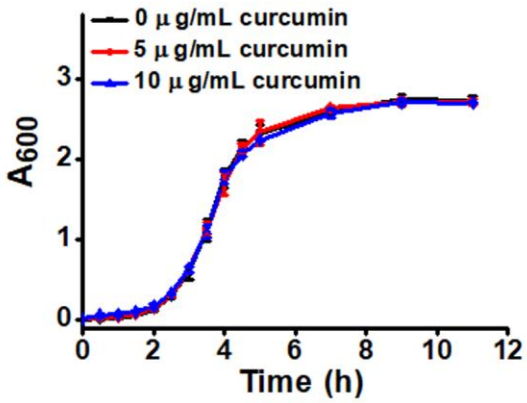

Fig. S8 The growth curve of *S. aureus* cells that were treated with different-concentration curcumin. The *S. aureus* cell without curcumin treatment was used as the control. The samples were taken at indicated time points to measure the absorbance at 600 nm.

References

1 N. W. Luedtke, R. J. Dexter, D. B. Fried and A. Schepartz, *Nat. Chem. Biol.*, 2007, **3**, 779.

2 Y. Yang, Y. Liang and C.Y. Zhang, *Anal. Chem*., 2017, **89**, 4055.