Supporting Information

Structural and Biochemical Characterization of *Staphylococcus aureus* Cysteine Desulfurase Complex SufSU

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Sequence alignments for *SaSufS* to cysteine desulfurases
**Figure S1.** Sequence alignment of type II and type I cysteine desulfurases, showing that *Sa*SufS is a type II cysteine desulfurase. Sequences were fetched using the BLAST tool and alignment was executed using Clustal Omega. The figure was generated in Jalview.

The top row is the *Sa*SufS sequence. The next 10 sequences in each row are from various type II cysteine desulfurases, and the bottom two sequences are type I cysteine desulfurases (*E. coli* IscS and *A. vinelandii* NifS). Percentages next to organism reflect the amount of identical residues to *Sa*SufS. Residues are colored blue based on ≥50% conservation between species, with higher conservation corresponding to darker shading.

Several important parts of the sequences are denoted with boxes and arrows. The red box shows where the flexible loop is present in type I cysteine desulfurases and absent in type II cysteine desulfurases. The green box shows where the β-hook is present in type II cysteine desulfurases and absent in type I cysteine desulfurases. Important conserved residues that are present in and around the active site are indicated with arrows, including a catalytic cysteine responsible for the formation of the persulfide intermediate (Cys389 in *Sa*SufS), a lysine that covalently harbors PLP (Lys250 in *Sa*SufS), and a histidine that acts as a base in the sulfur acquisition reaction from the cysteine substrate (His147 in *Sa*SufS).
Sequence alignments for \textit{SaSufU} to U-type proteins
**Figure S2.** Sequence alignment of U-type proteins, showing that *SaSufU* shares significant homology to this class of enzymes. Sequences were fetched using the BLAST tool and alignment was executed using Clustal Omega. The figure was generated in Jalview.

The top row is the *SaSufU* sequence. The next 10 sequences in each row are from various U-type proteins. Percentages next to organism reflect amount of identical residues to *SaSufU*. Residues are colored blue based on ≥50% conservation between species, with higher conservation corresponding to darker shading.

Several highly conserved residues are denoted with arrows. These residues are likely to coordinate Zn$^{2+}$ based on conservation in *BsSufU*. In *SaSufU*, these residues are Cys43, Asp45, Cys68, and Cys130, with Cys43 as the hypothesized residue that participates in persulfide transfer from *SaSufS*. 
PLP quantification in *SaSufS*

PLP in *SaSufS* was quantified using absorbance measurements. In these experiments, the PLP was released from its covalent attachment to Lys250 by the addition of NaOH, and the protein was precipitated by addition of HCl. We quantified the amount of PLP by comparing the absorption band of our samples at 390 nm (Figure S3) to a standard curve of free PLP (Figure S4), which has a signature absorption band at 390 nm.

The absorbances of the control and *SaSufS* samples at 390 nm are numerically summarized in Table S1. The *SaSufS* concentration in the sample was 43.5 μM, and PLP was measured to be 45.5 ± 1.1 μM by averaging triplicate measurements, thus yielding an occupancy of 104 ± 2%.

![Absorbance Spectra of Free PLP and PLP Extracted From SaSufS](image)

**Figure S3.** Absorbance spectra of free PLP in solution at varying concentrations and free PLP released from *SaSufS*. The steep rise around 310 nm corresponds to the tail of the protein peak at 280 nm.
Figure S4. Standard curve of free PLP absorbance at 390 nm.

Table S1. Absorbance values for free and extracted PLP at 390 nm.

| PLP Concentration (µM) | Absorbance       |
|------------------------|------------------|
| 0                      | 0.002 ± 0.0004   |
| 20                     | 0.102 ± 0.0033   |
| 40                     | 0.205 ± 0.0006   |
| 60                     | 0.314 ± 0.0003   |
| 80                     | 0.418 ± 0.0005   |
| 100                    | 0.531 ± 0.0005   |
| SaSufS (protein concentration = 43.5; measurement concentration = 45.5) | 0.239 ± 0.0004   |
\textbf{Zn}^{2+} \textit{quantitation in} \textit{SaSufU}

\textit{Zn}^{2+} \textit{in} \textit{SaSufU} \textit{was quantified using fluorescence measurements. In these experiments, the \textit{Zn}^{2+} \textit{was released from its coordination sphere by alkylation with iodoacetamide, and the released \textit{Zn}^{2+} \textit{was incubated with the selective fluorophore, FluoZin-3. We quantified the amount of \textit{Zn}^{2+} \textit{by comparing fluorescence (ex. 494 nm/em. 516 nm) of our samples to a standard curve of ZnCl}_2 \textit{(Figure S5).}}

The fluorescence of the control and \textit{SaSufU} samples are numerically summarized in Table S2. The \textit{SaSufU} concentration in the sample was 2.06 \textmu{M}, and \textit{Zn}^{2+} \textit{was measured to be 2.07 \pm 0.03 \textmu{M} by averaging triplicate measurements, thus yielding a 1:1 molar ratio of \textit{Zn}^{2+}:\textit{SaSufU}.}

\textbf{Figure S5.} Fluorescence standard curve of FluoZin-3-\textit{Zn}^{2+}.

\textbf{Table S2.} FluoZin-3-\textit{Zn}^{2+} \textit{data used to quantify \textit{Zn}^{2+} \textit{in} \textit{SaSufU}.}

\begin{center}
\begin{tabular}{|c|c|}
\hline
\textit{Zn}^{2+} \textit{Concentration (\textmu{M})} & \textbf{Fluorescence} \\
\hline
0 & 0 \pm 354 \\
2 & 10535 \pm 373 \\
4 & 21914 \pm 343 \\
6 & 33383 \pm 450 \\
8 & 42700 \pm 330 \\
\textit{SaSufU} (protein concentration = 2.06; measured concentration = 2.07) & 11262 \pm 187 \\
\hline
\end{tabular}
\end{center}
Cysteine desulfurase activity assays

Shown below are the data for each of the three plates characterizing cysteine desulfurase activity, each of which includes triplicate samples of each experiment. The standard range of alanine-NDA fluorescence required to quantify the cysteine desulfurase activity of both *B. subtilis* and *S. aureus* SufSU is best fit with a binomial curve (Figures S6-S8). Although the theoretical maximum alanine production from our assay parameters is 30 nmol, a standard curve characterizing slightly excess alanine is necessary to capture as much data as possible due to the variability in the fluorescence measurements. The raw fluorescence data is summarized for each plate (Tables S3, S6, and S9). The amount of alanine was calculated by taking the leftmost root of the quadratic formula and the values are summarized for each plate (Tables S4, S7, and S10). Stimulation of SufS by SufU was calculated by taking the ratio of the averaged alanine produced by SufS alone with the averaged alanine produced by SufSU for the respective intra- and interspecies analysis. Alanine production averages and stimulation are summarized for each plate (Tables S5, S8, and S11).

**Figure S6.** Fluorescence standard curve of alanine-NDA adduct for plate 1 with a binomial fit.

**Table S3.** Raw fluorescence data for triplicate measurements in plate 1.

| Sample | *SaSufS* | *SaSufSU* | *BsSufS* | *BsSufSU* | *SaSufS+BsSufU* | *BsSufS+SaSufU* |
|--------|----------|-----------|----------|-----------|----------------|-----------------|
| 1      | 11528    | 14385     | 10802    | 42187     | 12041          | 33852           |
| 2      | 11007    | 14368     | 8514     | 30193     | 11756          | 27495           |
| 3      | 10787    | 14390     | 9407     | 48015     | 11888          | 27251           |
Table S4. Calculated alanine for triplicate measurements in plate 1 using leftmost root of quadratic formula.

| Sample | SaSufS | SaSufSU | BsSufS | BsSufSU | SaSufSU + BsSufU | BsSufSU + SaSufSU |
|--------|--------|---------|--------|---------|------------------|-------------------|
| 1      | 4.12210845 | 6.804117 | 3.461062 | Error* | 4.594019 | 31.20472 |
| 2      | 3.64692699 | 6.787754 | 1.427053 | 25.26047 | 4.331347 | 21.46949 |
| 3      | 3.44748634 | 6.808931 | 2.212313 | Error* | 4.452849 | 21.14516 |

*Fluorescence value is out of range of curve – omitted from averages.

Table S5. Average and standard deviation of cysteine desulfurase activity for plate 1.

| Protein | Average nmol alanine | Standard Deviation | Stimulation Factor |
|---------|----------------------|--------------------|-------------------|
| SaSufS  | 3.73884059           | 0.34657586         | 0                 |
| SaSufSU | 6.80026744           | 0.01110087         | 1.81881716        |
| BsSufS  | 2.36680916           | 1.02576807         | 0                 |
| BsSufSU | 25.260474            | -                  | 10.6727971        |
| SaSufS+BsSufU | 4.45940515 | 0.1314585 | 1.19272406 |
| BsSufS+SaSufU | 24.6064545 | 5.71656336 | 10.3964675 |

Figure S7. Fluorescence standard curve of alanine-NDA adduct for plate 2 with a binomial fit.

Table S6. Raw fluorescence data for triplicate measurements in plate 2.

| Sample | SaSufS | SaSufSU | BsSufS | BsSufSU | SaSufSU + BsSufU | BsSufSU + SaSufSU |
|--------|--------|---------|--------|---------|------------------|-------------------|
| 1      | 9719   | 11679   | 9323   | 40815   | 11456            | 36186             |
| 2      | 9737   | 11908   | 8632   | 36755   | 9295             | 36642             |
| 3      | 9518   | 11480   | 8490   | 39025   | 9909             | 32459             |

Table S7. Calculated alanine for triplicate measurements in plate 2 using leftmost root of quadratic formula.
Table S8. Average and standard deviation of cysteine desulfurase activity for plate 2.

| Protein     | Average nmol alanine | Standard Deviation | Stimulation Factor |
|-------------|----------------------|--------------------|-------------------|
| SaSufS      | 3.6744995            | 0.14268129         | 0.0               |
| SaSufSU     | 6.08760661           | 0.25755136         | 1.65671723        |
| BsSufS      | 2.69032369           | 0.51887631         | 0.0               |
| BsSufSU     | 47.3437169           | 4.15573658         | 17.5977772        |
| SaSufS+BsSU | 4.34209922           | 1.31929823         | 1.18168453        |
| BsSufS+SaSU | 40.0702927           | 4.11202276         | 14.8942274        |

Figure S8. Fluorescence standard curve of alanine-NDA adduct for plate 3 with a binomial fit.

$$y = -9.4039x^2 + 1044.2941x + 6227.0851$$

$$R^2 = 0.9973$$

Table S9. Raw fluorescence data for triplicate measurements in plate 3.

| Sample | SaSufS | SaSufSU | BsSufS | BsSufSU | SaSufS+BsSU | BsSufS+SaSU |
|--------|--------|---------|--------|---------|-------------|-------------|
| 1      | 10615  | 11115   | 7994   | 30872   | 9934        | 26612       |
| 2      | 11009  | 10802   | 9025   | 30221   | 10136       | 30053       |
| 3      | 10656  | 11060   | 7866   | 28130   | 10897       | 27405       |

Table S10. Calculated alanine for triplicate measurements in plate 3 using leftmost root of quadratic formula.
Table S11. Average and standard deviation of cysteine desulfurase activity for plate 3.

| Sample |  SaSufS  |  SaSufSU  |  BsSufS  |  BsSufSU  |  SaSufS+BsSufU  |  BsSufS+SaSufU  |
|--------|---------|----------|---------|---------|----------------|-----------------|
| 1      |  4.37409018 |  4.896494 |  1.718567 |  34.02428 |  3.671042 |  25.27119 |
| 2      |  4.78529492 |  4.568842 |  2.747202 |  32.47052 |  3.878583 |  32.08626 |
| 3      |  4.41672629 |  4.838766 |  1.592229 |  28.06835 |  4.668066 |  26.69854 |

| Protein | Average nmol alanine | Standard Deviation | Stimulation Factor |
|---------|----------------------|--------------------|--------------------|
| SaSufS  | 4.52537046           | 0.22610839         | 0                  |
| SaSufSU | 4.76803389           | 0.17490323         | 1.05362289         |
| BsSufS  | 2.01933278           | 0.6335108          | 0                  |
| BsSufSU | 31.5210494           | 3.08940242         | 15.6096358         |
| SaSufS+BsSufU | 4.07256359 | 0.52605678 | 2.01678674 |
| BsSufS+SaSufU | 28.0186659 | 3.59421054 | 13.8752097 |