Electronic Supplementary Information

Competitive Profiling of Ligandable Cysteines in *Staphylococcus aureus* with an Organogold Compound

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Experimental Section

General

Solvents and reagents (reagent grade) were all commercially available and used without further purification. The enzymes were expressed and purified following the procedures below. All reagents were purchased from Sigma-Aldrich. The cyclometalated Au(III) complex [Au($C^{CO}N$)Cl_2] (**1**, $C^{CO}N$ = 2-benzoylpyridine) was synthesized according to previously published procedures.[1, 2] The compound's purity was assessed to be > 95%.

Cultivation and lysis of *S. aureus* SH1000

The cultivation and lysis of *Staphylococcus aureus* SH1000 was performed analogously to a published procedure.[3] *S. aureus* SH1000 was a kind gift from Simon J. Foster, The Krebs Institute, Department of Molecular Biology and Biotechnology, University of Sheffield.[4] Overnight cultures were inoculated with 5 µL of a glycerol stock into 5 mL of B medium (10 g/L peptone, 5 g/L NaCl, 5 g/L yeast extract, 1 g/L K_2HPO_4) and grown overnight (200 rpm, 37 °C). B medium was inoculated 1:100 with an overnight culture and incubated (200 rpm, 37 °C) until 1 h after it reached the stationary phase (OD ≈ 6). The cells were harvested by centrifugation (8,000 xg, 10 min, 4 °C), pellets of 100 mL initial culture were pooled and the pellets were washed two times with PBS (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 140 mM NaCl, 2.7 mM KCl, pH = 7.4) prior to the immediate use or storage at −80 °C. 5 mL PBS were added to the bacterial pellets and the pellets were resuspended and transferred into 7 mL tubes containing 0.1 mm ceramic beads (Peqlab, 91-PCS-CK01L). Cells were lysed in a Precellys 24 bead mill (3 × 30 s, 6,500 rpm) while cooling with an airflow that was pre-cooled with liquid nitrogen. The suspension was transferred into a microcentrifuge tube and centrifuged (20,000 xg, 30 min, 4 °C). The supernatant of several samples was pooled and filtered through a 0.45 µm filter. Protein concentration of the lysate was determined using a bicinchoninic acid (BCA) assay (typical concentrations were between ~2 mg/mL and ~3 mg/mL) and the concentration was adjusted to 1 mg/mL with PBS. The lysates were used immediately for all MS experiments.

Competitive isoDTB-ABPP Experiments

The chemoproteomic experiments were performed analogously to a published procedure.[3] 1.00 mL freshly prepared lysate of *S. aureus* SH1000 were incubated with 10 µL 100x Au($C^{CO}N$)Cl_2 in DMSO (*e.g.* 10 µL 10 mM stock for 100 µM final concentration) at room temperature for 1 h. Another 1.00 mL sample of lysate was incubated with 10 µL of DMSO. After this incubation, 20 µL of 50 mM IA-alkyne in DMSO were separately added to the lysate with and without competitor and incubated at room temperature for 1 h. The samples were clicked to the heavy (DMSO-treated) and light (compound-treated) isoDTB tags by adding 120 µL of a solution consisting of 60 µL 0.9 mg/mL TBTA ligand in 4:1 ^{t}BuOH/DMSO, 20 µL 12.5 mg/mL $CuSO_4 \cdot 5H_2O$ in ddH_2O, 20 µL 13 mg/mL TCEP in ddH_2O and 20 µL 5 mM of the respective isoDTB tag in DMSO. After incubation of the

samples at room temperature for 1 h, the light- and heavy-labeled samples were combined into 8 mL of cold acetone in order to precipitate all proteins. Precipitates were stored at –20 °C overnight.

MS Sample Preparation for isoDTB-ABPP

The MS samples were prepared according to a published procedure.[3] The protein precipitates were centrifuged at 3,500 rpm at 25 °C for 10 min. The supernatant was removed, and precipitates resuspended in 1 mL cold methanol by sonification. After centrifugation at 20,000 xg at 4 °C for 10 min, the supernatant was removed. This wash step with methanol was repeated one more time. The pellets were dissolved in 300 µL 8 M urea in 0.1 M triethylammonium bicarbonate (TEAB) by sonification. 900 µL 0.1 M TEAB were added to obtain a concentration of 2 M urea. This solution was added to 1.2 mL of washed high-capacity streptavidin agarose beads (50 µL initial slurry, Fisher Scientific, 10733315) in 0.2% nonyl phenoxypolyethoxylethanol (NP40) in PBS. The samples were rotated at room temperature for 1 h in order to assure binding to the beads.

The beads were centrifuged (1,000 xg, 1 min) and the supernatant was removed. The beads were resuspended in 600 µL 0.1% NP40 in PBS and transferred to a centrifuge column (Fisher Scientific, 11894131). Beads were washed two times with 600 µL 0.1% NP40 in PBS, three times with 600 µL PBS and three times with 600 µL ddH_2O. The beads were resuspended in 600 µL 8 M urea in 0.1 M TEAB, transferred to a Protein LoBind tube (Eppendorf) and centrifuged (1,000 xg, 1 min). The supernatant was removed, and the beads were resuspended in 300 µL 8 M urea in 0.1 M TEAB. 15µL of dithiothreitol (DTT; 31 mg/mL in ddH_2O) were added and the beads incubated at 37 °C with shaking at 200 rpm for 45 min. Free thiol groups were modified by adding 15 µL iodoacetamide (74 mg/mL in ddH_2O) and incubation at 25 °C for 30 min while shaking. Remaining iodoacetamide was quenched by adding 15 µL DTT (31 mg/mL in ddH_2O) and incubation at 25 °C for another 30 min while shaking.

For trypsin digestion, 900 µL 0.1 M TEAB were added to the samples after alkylation to obtain a urea concentration of 2 M. Samples were centrifuged (1,000 x g, 1 min) and the supernatant was removed. The beads were resuspended in 200 µL 2 M urea in 0.1 M TEAB. 4 µL 0.5 mg/mL trypsin (Promega, V5113) were added and samples incubated at 37 °C with shaking at 200 rpm overnight.

After the digestion, samples were diluted by adding 400 µL of 0.1% NP40 in PBS and transferred to a centrifuge column (Fisher Scientific, 11894131). Beads were washed three times with 600 µL 0.1% NP40 in PBS, three times with 800 µL PBS (MS-grade) and three times with 800 µL water (MS-grade). Peptides were eluted by adding 200 µL 0.1% trifluoroacetic acid in 50% acetonitrile in water to the column. 100 µL of the elution buffer were added two times more to the column and peptides eluted into a Protein LoBind tube (Eppendorf) by centrifuging at 3,000 xg for 3 min. The solvent was removed in a vacuum concentrator by rotating at 30 °C for approximately 5 h. Samples were dissolved in 30 µL 1% trifluoroacetic acid in water by sonification for 5 min. Samples were filtered (Merck, UVC30GVNB) washed with the same solvent and transferred into MS sample vials. Samples were stored at –20 °C until measurement.

Sample Analysis for isoDTB-ABPP by LC-MS/MS

The samples were analyzed analogously to a previously published procedure.[3] 5 µL of the samples were analyzed using a Qexactive Plus mass spectrometer (ThermoFisher) coupled to an Ultimate 3000 nano HPLC system (Dionex). Samples were loaded on an Acclaim C18 PepMap100 trap column (75 µm ID × 2 cm, Acclaim, PN 164535) and washed with 0.1% trifluoroacetic acid. The subsequent separation was carried out on an AURORA series AUR2-25075C18A column (75 µM ID × 25 cm, Serial No. IO257504282) with a flow rate of 400 nL/min using buffer A (0.1% formic acid in water) and buffer B (0.1% formic acid in acetonitrile). The column was heated to 40 °C. Analysis started with washing in 5% B for 7 min followed by a gradient from 5% to 40% buffer B over 105 min, an increase to 60% B in 10 min and another increase to 90% B in 10 min. 90% B was held for 10 min, then decreased to 5% in 0.1 min and held at 5% for another 9.9 min. The Qexactive Plus mass spectrometer was run in a TOP10 data-dependent mode. In the orbitrap, full MS scans were collected in a scan range of 300-1500 m/z at a resolution of 70,000 and an AGC target of 3e6 with 80 ms maximum injection time. The most intense peaks were selected for MS2 measurement with a minimum AGC target of 1e3 and isotope exclusion and dynamic exclusion (exclusion duration: 60 s) enabled. Peaks with unassigned charge or a charge of +1 were excluded. Peptide match was “preferred”. MS2 spectra were collected at a resolution of 17,500 aiming at an AGC target of 1e5 with a maximum injection time of 100 ms. Isolation was conducted in the quadrupole using a window of 1.6 m/z. Fragments were generated using higher-energy collisional dissociation (HCD, normalized collision energy: 27%) and finally detected in the orbitrap.

isoDTB-ABPP Data Evaluation using MaxQuant

A FASTA database for *S. aureus* SH1000 was downloaded from www.uniport.org using a search for “93061” as “Taxonomy [OC]” at UniProtKB on 27.02.2018. This corresponds to the FASTA database for the strain NCTC8325. Comparative sequencing of these two strains has been reported[5] and the respective changes have been manually made to the FASTA file. These include several point mutations, the deletion of the partial proteins Q2FWJ0 and

Q2FWJ1 as well as the addition of the rsbU gene from *S. aureus* Newman (A0A0H3KE27). The reverse sequences were manually added to the FASTA database.

Quantification with MaxQuant[6] was performed analogously to a published procedure utilizing a workaround to also allow quantification of peptides that contain one or more carbamidomethylated cysteines in addition to the isoDTB tag-labelled cysteine.[3] Briefly, in this workaround "U", which normally stands for selenocysteine, is used as a placeholder amino acid for the modified cysteine. To accomplish this, all selenocysteine-containing proteins were deleted from the FASTA databases; which were usually very few. Afterwards, each cysteine in the FASTA database was individually replaced with a "U" generating n different sequences with a single "U" for a protein with n cysteines. For each individual replacement, an entry in the FASTA database was created, which was named in the format "UniProt code"_"C""number of the cysteine". The unmodified sequence was deleted from the FASTA database, except if the protein did not contain any cysteine, in which case the unmodified entry was renamed to "UniProt Code" _"C0" and kept in the database. In this way, for each cysteine in the database, a unique sequence was created, in which it was marked as the modified cysteine (by being replaced by the placeholder "U") and all other cysteines were marked as unmodified (were remaining "C" in the database). In this way, there was always only one modified cysteine in each peptide to be detected and quantified.

MS raw data were analyzed using MaxQuant software[6] (version 1.6.17.0). Standard settings were used with the following changes and additions: The modified FASTA databases with individual substitutions of cysteines with the placeholder "U" were used. Labels were set on the placeholder amino acid "U" for the light isoDTB tag as light label ($C_{28}H_{46}N_{10}O_6S_1Se_{-1}$) and the heavy isoDTB tag as heavy label ($C_{24}{}^{13}C_4H_{46}N_8{}^{15}N_2O^6S_1Se_{-1}$). A multiplicity of 2 was set and a maximum number of labelled amino acids of 1. The digestion enzyme was set to Trypsin/P with a maximum number of missed cleavages of 2. No variable modifications were included. The "Re-quantify" option was enabled. Carbamidomethyl (C_2H_3NO) was used as fixed modification on cysteine. Contaminants were included. Peptides were searched with a minimum peptide length of 7 and a maximum peptide mass of 4,600 Da. "Second peptides" and "Dependent peptides" were disabled and the option "Match between run" was enabled with a Match time window of 0.7 min and an alignment window of 20 min. An FDR of 0.01 was used for Protein FDR, PSM FDR and XPSM FDR.

The "peptides.txt" file of the MaxQuant analysis was used for further analysis. All peptide sequences without a modified cysteine (placeholder "U") and all reverse sequences were deleted. Only the columns "Sequence", "Leading Razor Protein", "Start Position" and the columns for "Ratio H/L" for both replicates were kept. The "Leading Razor Protein" was renamed to the UniProt Code without the indicator for the number of the cysteine. All individual ratios were filtered out if they were "NaN" and all other values were transformed into the $\log_2$-scale. For each peptide, the data was filtered out, if it was not present in both replicates or if the standard deviation between the replicates exceeded a value of 1.41. For each peptide, an identifier was generated in the form "UniProt Code"_"C""residue number of the modified cysteine". The data for the same replicate for all peptides with the same identifier, and therefore the same modified cysteine, were combined. Here, the median of the data was used. The data was filtered out if the standard deviation exceeded a value of 1.41. Each modified cysteine was kept in the dataset once with the shortest peptide sequence as the reported sequence. For each modified cysteine, the values of the two replicates were combined, but the individual values were also reported. The values were combined as the median and the data was filtered out if there was only data in one of the replicates or if the standard deviation exceeded a value of 1.41. These are the final ratios that are reported. The information on the "Gene Name" and "Name" was linked back from the FASTA database.

Downstream Data Analysis for isoDTB-ABPP experiments

The isoDTB-ABPP data was further analyzed analogously to published procedure[3] using the Perseus software.[7] All individual values for each modified cysteine for the same condition were loaded into Perseus and analyzed using a one-sample t-test against a value of $\log_2(R) = 0$. Conditions with $p < 0.05$ were considered significant and compounds were deemed to engage a certain cysteine at a specific condition and called a "hit" if the statistical significance was $p < 0.05$ and the media ratio was $\log_2(R) > 2$ according to the values described under "isoDTB-ABPP Data Evaluation using MaxQuant". For volcano plots, the median values for $\log_2(R)$ described under "isoDTB-ABPP Data Evaluation using MaxQuant" and the $-\log_{10}(p)$-values derived from Perseus are used.

Data availability

All proteomics raw data, the parameter and result files of MaxQuant and the used modified FASTA databases have been deposited to the ProteomeXchange[8] Consortium *via* the PRIDE partner repository[9] with the dataset identifier project accession: PXD031708.

Analysis of Protein Essentiality and of Functional Sites

The analysis of protein essentiality and of functional sites within the proteins was done analogously to a previous procedure.[3, 10, 11]

Cloning

All cloning experiments were conducted according to a published procedure.[3] N-terminal 6xHis-tagged (Uniprot ID: Q2G041) and C-terminal 6xHis-tagged (Uniprot ID: Q2FVA6) constructs (see table) of *S. aureus* SH1000 were cloning into pET28a+ expression vectors using standard techniques based on polymerase chain reaction (PCR), restriction, digestion and ligation.

UniProt ID	primer	sequence (5´→3´)
Q2G041	forward	CTGCAGCATATGACTGAAATAGATTTTGATATAGCAA
	reverse	GTTAGCGGATCCTTAAGCTTGATCGTTTAAATGTTCAATA
Q2FVA6	forward	CTGCAGCCATGGGCAGTAACCTTGAAATCAAACAAGGC
	reverse	GTTAGCGCGGCCGCACCAAGATATACATCTTGATATGAATC

Genomic DNA was extracted from 2 mL *S. aureus* SH1000 culture (OD_{600} = 1) with a Bacterial DNA Kit (peqlab) and a Nucleo Spin Microbial DNA Kit (Macherey-Nagel). PCRs were carried out in a CFX96 Real-time System in combination with a C1000 Thermal Cycler (Biorad). The PCR mixture contained 10 µL GC or HF buffer (NEB), 1 µL dNTP mix (10 mM), 2.5 µL forward primer (10 µM, Q2G041: 5´-CTGCAGCATATGACTGAAATAGATTTTGATATAGCAA-3´;
Q2FVA6: 5´-CTGCAGCCATGGGCAGTAACCTTGAAATCAAACAAGGC-3´), 2.5 µL reverse primer (10 µM, Q2G041: 5´-GTTAGCGGATCCTTAAGCTTGATCGTTTAAATGTTCAATA-3´;
Q2FVA6: 5´-GTTAGCGCGGCCGCACCAAGATATACATCTTGATATGAATC-3´), 1 µL genomic DNA as template (0.1-1 ng), 0.5 µL Phusion High Fidelity DNA polymerase (NEB) and 32.5 µL ddH_2O. After initial denaturation (98 °C, 30 s), the mixtures underwent 35 cycles of denaturation (98 °C, 10 s), annealing (Q2G041: 68 °C, Q2FVA6: 72 °C, 20 s) and extension (72 °C, Q2G041 and Q2FVA6: 57 s), before a final extension (72 °C, 10 min). PCR products were purified by agarose gel electrophoresis on a 1% agarose gel run at 90 V for 50 min. After extraction with an E.Z.N.A Gel Extraction Kit (Omega bio-tek), 500 ng of the PCR products were digested in Cut Smart buffer (NEB) in a total volume of 50 µL at 37 °C for 90 min using 5 units of the respective restriction enzymes (Q2G041: NdeI and BamHI; Q2FVA6: NcoI and NotI), each, which were then inactivated by incubation at 80 °C for 20 min. Isolation of the digest was carried out using an E.Z.N.A Gel Extraction Kit (Omega bio-tek). pEt28a+ vector was extracted from NEB5α *E. coli* using a NucleoSpin Plasmid EasyPure kit (Macherey-Nagel). 500 ng vector was digested in Cut Smart buffer (NEB) in a total volume of 50 µL at 37 °C for 1 h using 5 units of the respective restriction enzymes, each. In order to dephosphorylate the ends the restriction sites, 5 units Antarctic phosphatase (NEB) in Antarctic phosphates buffer (NEB) were added and digestion was continued for another 30 min before heat inactivation at 80 °C for 20 min. Purification of the digested vector was carried out by agarose gel electrophoresis and gel extraction. 14 fmol digested pET28a+ vector and 42 fmol digested insert were ligated using 1 µL Quick Ligase (NEB) in Quick Ligase buffer (NEB) in a total volume of 20 µL. The ligation mixture was incubated at room temperature for 5 min and 5 µL of the ligation products were subsequently transformed into XL1-blue chemically competent cells (Agilent). For this, 50 µL competent cells were thawed on ice, cautiously mixed with the respective DNA and kept on ice for 30 min. Afterwards, the cells were heat-shocked at 42 °C for 45 s and chilled on ice for 2 min. 500 µL SOC (super optimal broth with catabolite repression) medium were added and the cells incubated at 37 °C, 200 rpm for 1 h. The cells were harvested by centrifugation (6,000 xg, 2 min), resuspended in SOC medium and plated an LB agar plates containing 250 µg/mL kanamycin. For preparation of the plasmids, 5 mL of LB medium containing 250 µg/mL kanamycin were inoculated with single colonies of the transformation and incubated at 37 °C overnight. Plasmid DNA was prepared using a NucleoSpin Plasmid EasyPure kit (Macherey-Nagel) according to the manufacturer´s instructions and sequenced by GeneWiz.

Site-Directed Mutagenesis

The QuickChange site-directed mutagenesis system (Agilent) was used to construct the respective point mutants starting from the isolated wildtype plasmid DNA (see table).

UniProt ID	primer	sequence (5´→3´)
Q2FVA6_C75A	forward	GAAAATTATTGCCTCAGCGTCATTTGCCAAACATATGTTAG
	reverse	CTAACATATGTTTGGCAAATGACGCTGAGGCAATAATTTTC

The PCR mixture contained 10 µL GC buffer (NEB), 1 µL dNTP mix (10 mM), 1 µL forward primer (10 µM, Q2FVA6_C75A: 5´-GAAAATTATTGCCTCAGCGTCATTTGCCAAACATATGTTAG-3´), 1 µL reverse primer (10 µM, Q2FVA6_C75A: 5´-CTAACATATGTTTGGCAAATGACGCTGAGGCAATAATTTTC-3´),

1 µL template (0.1-1 ng), 0.5 µL Phusion High Fidelity DNA polymerase (NEB), 1-3 µL DMSO and 34.5-32.5 µL ddH_2O. After initial denaturation (95 °C, 30 s), the mixtures underwent 19 cycles of denaturation (95 °C, 10 s), annealing (55 °C, 30 s) and extension (72 °C, 5 min), before a final extension (72 °C, 10 min). 10 µL of the PCR mixture were digested with 20 units DpnI at 37 °C for 4 h before transformation into XL1-blue cells was carried out as described above. Plasmid DNA was prepared using a NucleoSpin Plasmid EasyPure kit (Macherey-Nagel) according to the manufacturer´s instructions and sequenced by GeneWiz.

Expression and Purification of Recombinant Proteins

The expression and purification of the described recombinant proteins were conducted according to a previous literature report.[3] Chemically competent *E. coli* BL21(DE3) cells were transformed with the respective plasmid following the protocol given in the section “Cloning”. For protein overexpression, 20 mL LB medium containing 250 µg/mL kanamycin were inoculated with single colonies or cryostocks of transformed *E. coli* BL21(DE3) cells and incubated at 37 °C, 200 rpm. The proteins were expressed overnight at 18 °C after induction at OD_{600} = 0.6 with 1 mM *isopropyl*-β-d-thiogalactoside. Cells were harvested (6,000 rpm, 10 min, 4 °C), washed with 30 mL PBS, resuspended in 20 mL wash buffer 1 (20 mM Tris-HCl, pH = 8.0, 150 mM NaCl, 10 mm imidazole) and lysed by sonication (Bandelin Sonoplus) under constant cooling with ice. Lysate was cleared by centrifugation (18,000 rpm, 30 min, 4 °C) and the supernatant was purified.

Crude soluble lysate was loaded onto 2 mL of Ni-NTA-agarose beads (Qiagen) equilibrated with wash buffer 1 and flow-trough was collected. The column was sequentially washed with 10 column volumes of wash buffer 1, 10 column volumes of wash buffer 2 (20 mM Tris-HCl, pH = 8.0, 1 m NaCl, 10 mm imidazole) and 10 column volumes of wash buffer 3 (20 mM Tris-HCl, pH = 8.0, 150 mM NaCl, 20 mM imidazole). The proteins were eluted in 5 mL fractions using elution buffer (20 mM Tris-HCl, pH = 8.0, 150 mM NaCl, 300 mM imidazole). 50 mL of each collected fraction were mixed with 50 mL 2x Laemmli buffer and analyzed by SDS-PAGE with Coomassie staining. Fractions that contained the purified protein were concentrated using centrifugal filters (Merck) with a 10 kDa cut-off (Q2G041), respectively with a 3 kDa cut-off (Q2FVA6 and Q2FVA6_C75A). During the concentration procedure, elution buffer was exchanged for a storage buffer (20 mM Tris-HCl, pH = 8.0). Purity of the isolated proteins was furthermore analyzed by intact protein MS (IPMS).

Thioredoxin Reductase Activity Assay

The DTNB-coupled thioredoxin reductase activity assay based on the work by Lu et al.[12] was established and modified for our purposes by Schmidt et al.[13] Thioredoxin reductases (TrxR) from *S. aureus* SH1000 was expressed and purified according to the before mentioned methods. The substrate thioredoxin (Trx) from *E. coli* recombinant was purchased from Sigma-Aldrich (CAS: 52500-60-4). Stock solutions were prepared by dilution with TE buffer to achieve a concentration of 0.436 mg protein/mL for the enzymes, and 0.100 mg protein/mL for the substrate. TE buffer pH 7.5 was prepared (Tris-HCl 50 mM; EDTA 1 mM) in aqueous solution and pH was adjusted with NaOH 1M. The gold compound was freshly dissolved in DMSO (10^{-2} M stock solution) and diluted with TE buffer to reach the necessary concentrations.

Aliquots of the TrxR enzyme solution (10 µL), Trx substrate solution (10 µL), and NADPH (200 mM) in TE buffer (100 µL) were mixed in a well either containing solutions of the gold compound (20 µL) in increasing concentrations or only DMSO in buffer. The resulting solutions were incubated under moderate shaking for 75 min at 25 °C in a 96-well plate (final concentrations of DMSO: 0.5% v/v). Afterwards, 100 µL of reaction mixture (containing 200 mM NADPH and 5 mM DTNB in TE buffer solution) were added to each well, and thereby the conversion of DTNB to 5-TNB was initiated and monitored with a microplate reader (Tecan infinite M nano+) at 412 nm, ten times in 35 sec intervals for about 6 min.

The values were corrected using the absorbance of the blank solution. The increase in 5-TNB concentration over time followed a linear trend ($r^2 \geq 0.990$), and the enzymatic activities were calculated as the slopes (increase in absorbance per second) thereof. For each tested compound, non-interference with the assay components was confirmed by a negative control experiment, in which the highest test compound concentration was used and the aliquot of enzyme solution was replaced by the same amount of TE buffer. Furthermore, the reduction ability of Trx without the enzyme and TrxR without the substrate was evaluated. Both proteins are not able to reduce DTNB without their counterpart present. IC_{50} values were calculated as the concentration of the compound decreasing the enzymatic activity of the positive control by 50%, and are given as the means and error of three repeated experiments. (Figure S2)

Sample preparation for Intact Protein Mass Spectrometry Studies

GCN-5 like putative *N*-acetyltransferase (Uniprot code Q2FVA6) stock solutions in storage buffer (20 mM Tris-HCl, pH = 8.0) were defrosted and diluted with a mixture of H_2O/ACN (2:1) to a final concentration of 5 µM (500 µL). [Au($C^{CO}N$)Cl_2] was freshly dissolved in DMSO at a concentration of 15 mM and further diluted with a mixture of H_2O/ACN (2:1) to 150 µM prior the experiment. To achieve a molar ratio between the gold complex **1** and the GCN-5 like putative *N*-acetyltransferases of 3:1, 50 µL of the stock solution of the gold complex were added to 500 µL of

the respective enzyme solution (final volume 550 µL). Samples were incubated at room temperature (20°C) for 1 hour.

Intact Protein Mass Spectrometry Studies

The formation of either the cysteine arylation product or the gold adduct was assessed by high resolution mass spectrometry. Samples were separated by a UHPLC UltiMate 3000 (Thermo Scientific) and analyzed with a LTQ Orbitrap mass spectrometer (Thermo Scientific) by using a protein Xbridge® C18 3.5µm column (4.6*100mm), equipped with a Xbridge® BEH C18, 3.5µm precolumn (VanGuard® Cartidge, 3/PK 3.9 mm x 5 mm), both from Waters. The instrumental parameters for high-pressure liquid chromatography mass spectrometry (HPLC-HESI-MS) were as follows: sheath gas flow 30.00, aux gas flow: 15.00, positive polarity, 4.00 kV source voltage, 100.00 uA source current, 31.0 V capillary voltage, flow rate 0.3 ml/min, m/z range 300-2000.

HPLC method for measurements: 1 min 94% H_2O (0.1% formic acid) + 6% ACN (0.1% formic acid) pre-run, 1 min 94% H_2O (0.1% formic acid) + 6% ACN (0.1% formic acid) + 1.5 min gradient from 94% H_2O (0.1& formic acid) + 6% ACN (0.1% formic acid) to 5% H_2O (0.1% formic acid) + 95% ACN (0.1% formic acid) + 2 min 5% H_2O (0.1% formic acid) + 95% ACN (0.1% formic acid) + 15 sec gradient from 5% H_2O (0.1% formic acid) + 95% ACN (0.1% formic acid) to 94% H_2O (0.1% formic acid) + 6% ACN (0.1% formic acid) + 15 sec 94% H_2O (0.1% formic acid) + 6% ACN (0.1% formic acid).

HPLC method for cleaning: 15 sec 94% H_2O (0.1% formic acid) + 6% ACN (0.1% formic acid) pre-run, 30 sec 94% H_2O (0.1% formic acid) + 6% ACN (0.1% formic acid) + 15 sec gradient from 94% H_2O (0.1& formic acid) + 6% ACN (0.1% formic acid) to 0% H_2O (0.1% formic acid) + 100% ACN (0.1% formic acid) + 3.5 min 0% H_2O (0.1% formic acid) + 100% ACN (0.1% formic acid) + 15 sec gradient from 0% H_2O (0.1% formic acid) + 100% ACN (0.1% formic acid) to 94% H_2O (0.1% formic acid) + 6% ACN (0.1% formic acid) + 30 sec 94% H_2O (0.1% formic acid) + 6% ACN (0.1% formic acid).

Figures

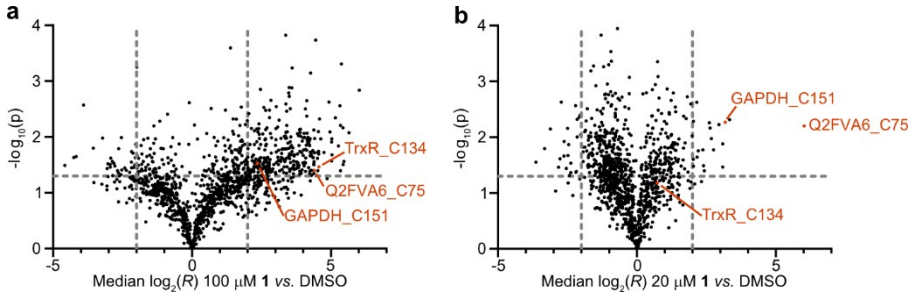

Figure S1. **(a,b)** Volcano plots of the isoDTB-ABPP experiments that show the median $\log_2(R)$ of the ratio between the heavy (compound-treated) and light (DMSO-treated) channels and the $-\log_{10}(p)$ of the statistical significance in a one-sample t-test for all quantified cysteines for compound **1** at 100 µM **(a)** and 20 µM **(b)**. Grey dotted lines indicate the cut-offs of $\log_2(R) = \pm 2$ and $p < 0.05$ that were used for hit selection. Selected proteins discussed in the text are highlighted in orange.

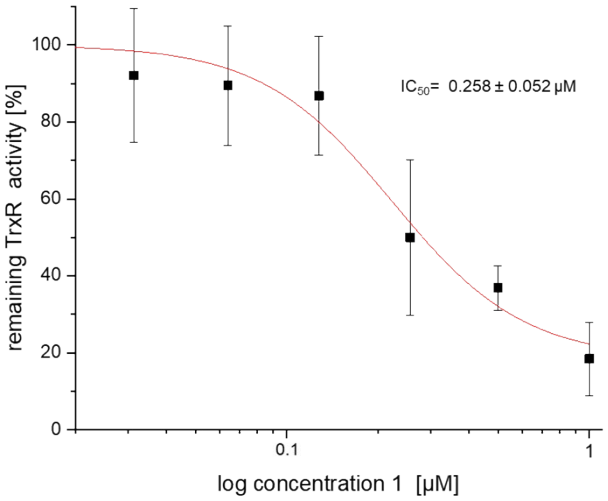

Figure S2. TrxR inhibition assay. The purified thioredoxin reductase wildtype of *S. aureus* SH1000 was incubated with **1** at different concentrations (0.03125 µM – 1.000 µM) for 75 min at 25°C in the presence of thioredoxin. The remaining TrxR activity was quantified by DTNB conversion for each test concentration of **1**. Data were processed with Origin using the Hill1 fit.

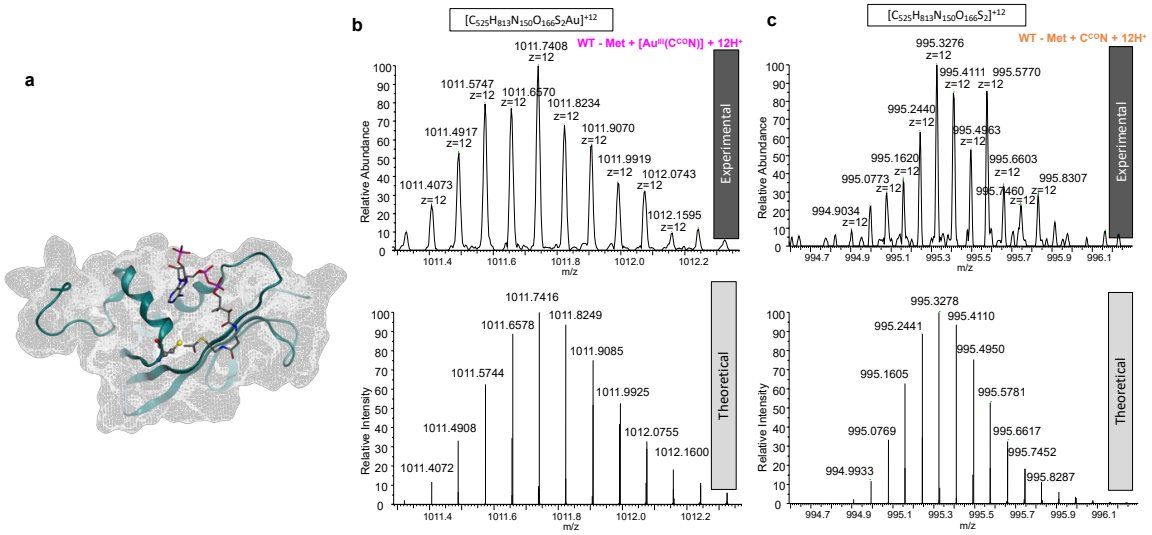

Figure S3. (**a**) Structure of the GCN5-like putative N-acetyltransferase (PDB: 2H5M). The ligandable Cys-75 is highlighted. The cofactor acetyl CoA is shown as sticks. (**b,c**) Comparisons of experimental vs. theoretical isotopic pattern distributions of the formed coordination (WT-Met-[$Au^{III}(C^{CO}N)$]) (**b**) and covalent (WT-Met-[($C^{CO}N$)], cysteine arylation product) (**b**) adducts.

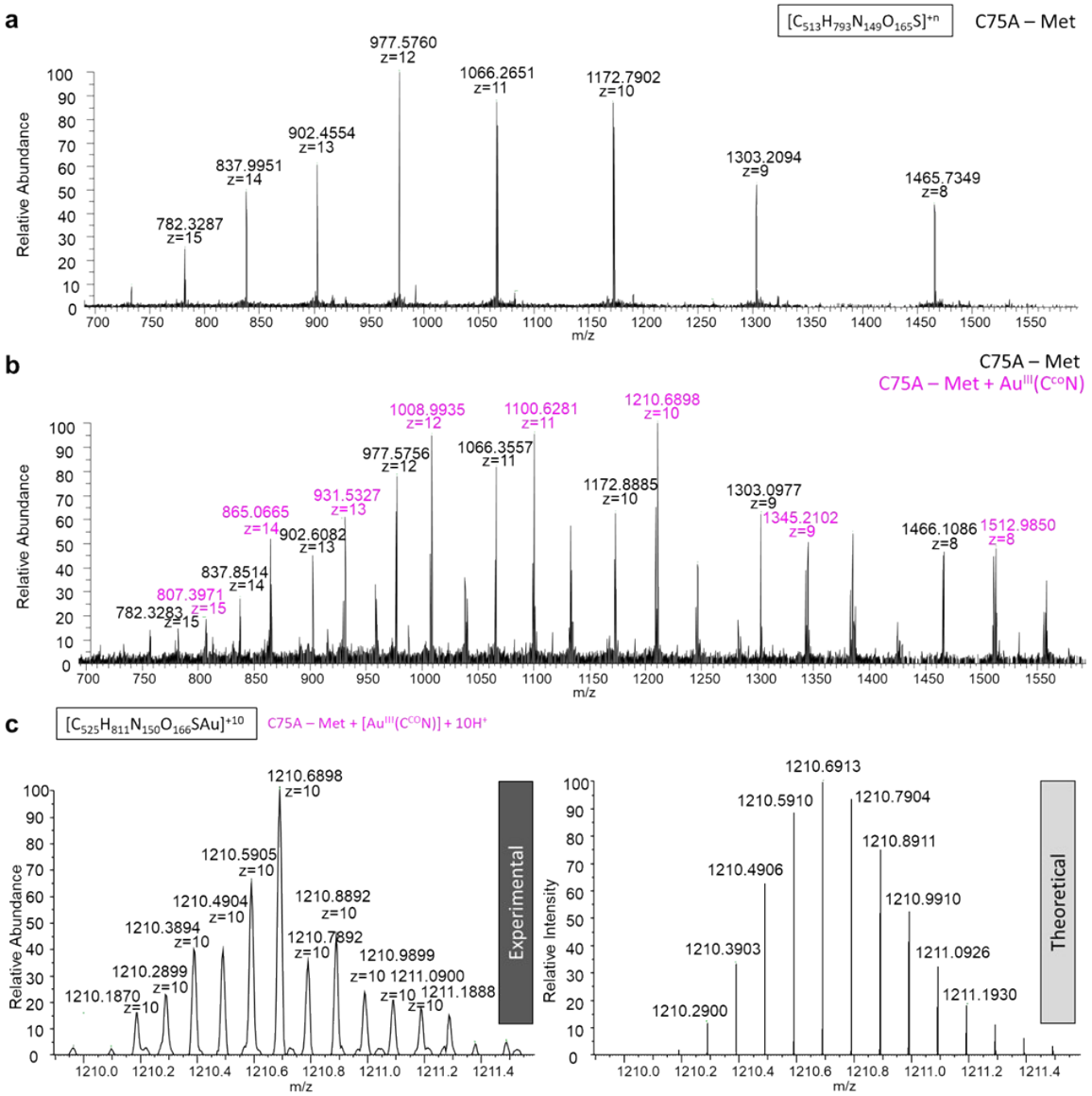

Figure S4. HR-MS spectra of GCN5-like putative *N*-acetyltransferase (C75A mutant) before (**a**) and after addition of [Au(C^{CO}N)Cl$_2$] incubated for 1 hour at room temperature (**b**); experimental vs theoretical isotopic pattern distribution of the C75A mutant-Au^{III}(C^{CO}N) adducts (**c**) + (**d**).

Tables

Table S1. All evaluated proteomics data of the isoDTB-ABPP experiments. This table can be found as additional file named “Supplementary Table 1.xlsx”.

Table S2. Experimental (M_{exp}) and theoretical (M_{theor}) masses of the HPLC-HR-MS experiments of the WT GCN-5 like putative *N*-acetyltransferase and of its C75A mutant.

Protein (Q2FVA6)	**Species**	**Abundance**	**M_{exp} [m/z]**	**M_{theor} [m/z]**	**Δppm**
SAOUHSC 02827	$[WT–Met+12H]^{12+}$	100%	980.3231	980.3229	0.20
SAOUHSC 02827(C75A)	$[C75A–Met+10H]^{10+}$	100%	1172.7902	1172.7886	1.36

Table S3. Experimental (M_{exp}) and theoretical (M_{theor}) masses of the detected species during the HPLC-HR-MS experiments of the WT as well as of the C75A mutant of the GCN-5 like putative *N*-acetyltransferase, incubated with compound **1** for 1 hour at 20°C.

Protein (Q2FVA6)	**Species**	**Abundance**	**M_{exp} [m/z]**	**M_{theor} [m/z]**	**Δppm**
SAOUHSC 02827	$[WT–Met+Au^{III}(C^{CO}N)+12H]^{12+}$	100%	1011.7408	1011.7416	0.79
	$[WT–Met+(C^{CO}N)+12H]^{12+}$	28%	995.3276	995.3278	0.20
SAOUHSC 02827(C75A)	$[C75A–Met+Au^{III}(C^{CO}N)+10H]^{10+}$	100%	1210.6898	1210.6913	1.24
	$[C75A–Met+11H]^{10+}$	84%	1172.8885	1172.8894	0.77

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