SUPPORTING INFORMATION

Structural basis of *Staphylococcus aureus* surface protein SdrC

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MATERIALS AND METHODS

Cloning of SdrC N2N3 and SdrC N2

The fragments corresponding to residues 178-335 (SdrC N2) and 178-496 (SdrC N2N3) of SdrC were amplified by PCR from *S. aureus* Newman genomic DNA. The PCR products were verified by agarose gel electrophoresis and purified using PCR product purification kit (Shanghai Sangon). The purified products were cloned into pET28a vector to generate expression plasmids pET28a-SdrC N2N3 and pET28a-SdrC N2. The successful construction of the constructs was confirmed by DNA sequencing. Oligonucleotide primers were listed in Table S1.

Protein expression and purification

For recombinant protein expression, plasmids pET28a-SdrC N2N3 and pET28a-SdrC N2, were transformed into *E. coli* BL21(DE3). Starter cultures were diluted 1:100 in LB medium containing kanamycin (50μg/ml) and incubated at 310 K with shaking until optical density reach to 0.6-0.8. 0.5 mM Isopropyl β-D-1-thiogalactopyranoside was added to induce protein expression and growth was continuing for 12 hours at 291 K.

Bacteria were then harvested by centrifugation and resuspended in lysis buffer (0.5 M NaCl, 5% glycerol, 10 mM Tris-HCl pH 7.4, 1 mM DTT). The lysate was centrifuged to remove cellular debris after homogenizing by sonification on ice. The supernatant was applied to a 5 ml nickel-charged HiTrap column (GE Healthcare) and the column was washed by 20 ml buffer (0.5 M NaCl, 5% glycerol, 10 mM Tri-HCl pH 7.4, 1 mM DTT, 50 mM imidazole). Ultimately, the protein was eluted in lysis buffer containing 500 mM imidazole. The eluants were further purified by an Sephadex 200 gel filtration column in Tris buffer (100 mM NaCl, 10 mM Tris-HCl pH 7.4, 1 mM DTT) after digesting with HRV3C protease at 277 K overnight. Fractions containing the target protein were then collected and concentrated to 30 mg/ml.

For site-specific mutagenesis of SdrCN2, rolling-circle PCR (using the primers listed in Table S2) was performed by following the standard procedures of the QuickChange Site-Directed Mutagenesis Kit (Stratagene). The successful construction of the mutated plasmids was confirmed by sequencing. The mutant proteins were purified by using the

same procedures as that of the wild-type protein.

Size-exclusion chromatography

The SdrCN2 wild-type and mutant proteins (after 6XHis tag cleavage) were subjected to size-exclusion chromatography analysis by using a HiLoad 16/600 Superdex 200 pg column (GE Healthcare) in the elution buffer (10 mM Tris·HCl pH 7.4, 100 mM NaCl) at a flow rate of 1 mL/min with UV detection at 280 nm.

Crystallization

Initial screening was performed at 16 ℃ in 48-well plates by setting drop vapor diffusion method using four screen kits from Hampton Research (Index reagent kits , Crystal Screen reagent kits, PEGRX reagent kits, and PEG/ion reagent kits)(*1-4*). The protein was crystallized by mixing 1.0 μl protein solution with 1.0 μl reservoir solution and equilibrating against 100μl reservoir solution. After optimizing the conditions through the variation of precipitant concentration, pH, and additives, the Ca^{2+}-mediated SdrC crystals were obtained in the condition of CS46 (0.2M Calcium acetate hydrate, 0.1M Sodium cacodylate trihydrate pH 6.5, 18% Polyethylene glycol 8000), and the crystals of SdrC without Ca^{2+} were obtained in the condition of Index36 (0.1M HEPES pH 7.0, 30% Jeffamine ED-2001 pH 7.0).

Structural data collection and determination

For data collection, crystals of SdrC N2N3 were flash frozen with a stabilizing solution containing 20% glycerol in a 100 K nitrogen gas stream. Data were collected at Shanghai Synchrotron Radiation Facility (SSRF) at a wavelength of 0.9785 Å using a MAuR225 (MAR Research, Hamburg) CCD detector at 100 K and processed with HKL3000(*5*). Further processing was carried out using programs from the CCP4 suite(*6*). The structure was solved by molecular replacement using the *S. aureus* UafA structure (PDB: 3IRP) as a search model and further refined with the PHENIX packages(*7*). Statistics of data collection and refinement are summarized in table S2

SUPPORTING FIGURES

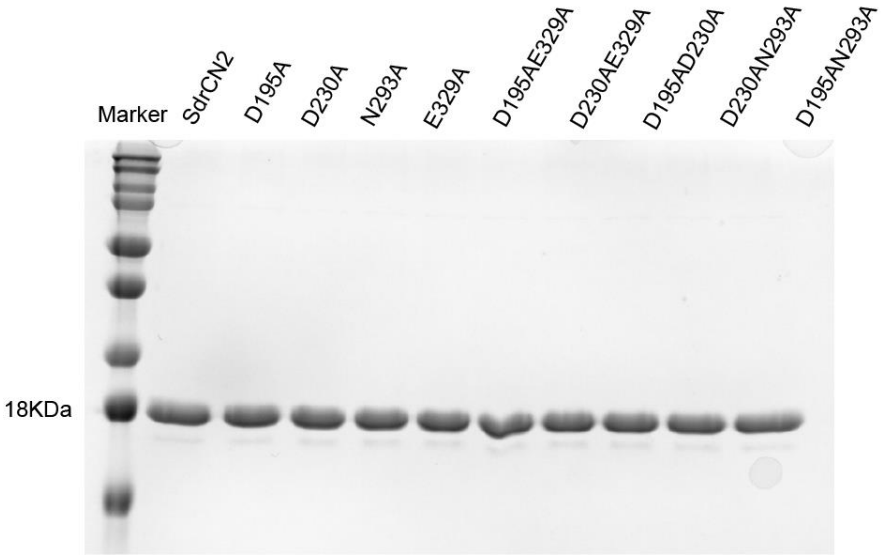

Figure S1. SDS-PAGE analysis of SdrCN2 and its mutants. The molecular weight of the protein is indicated on the diagram. The purity and stability of SdrCN2 proteins and its mutants can be detected by the SDS-PAGE gel.

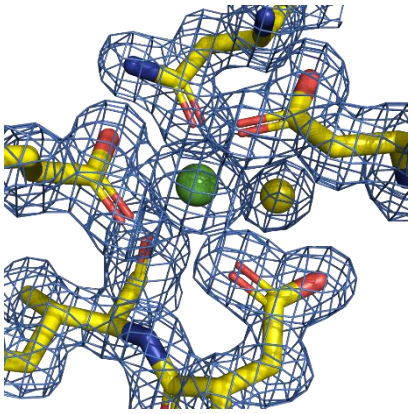

Figure S2. The electron density map of calcium ion chelation sites of SdrC. Detailed view of the electron density of SdrC is shown as a blue mesh.

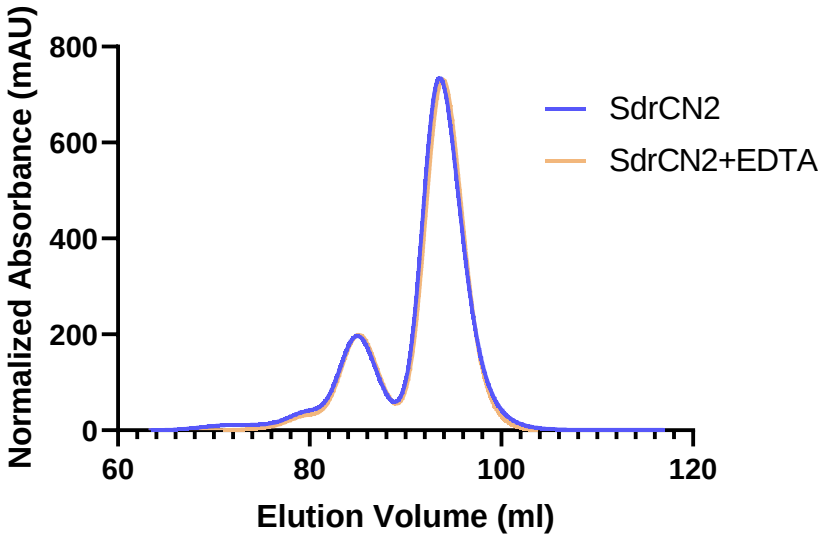

Figure S3. Normalized gel chromatogram of SdrCN2 protein with and without EDTA.

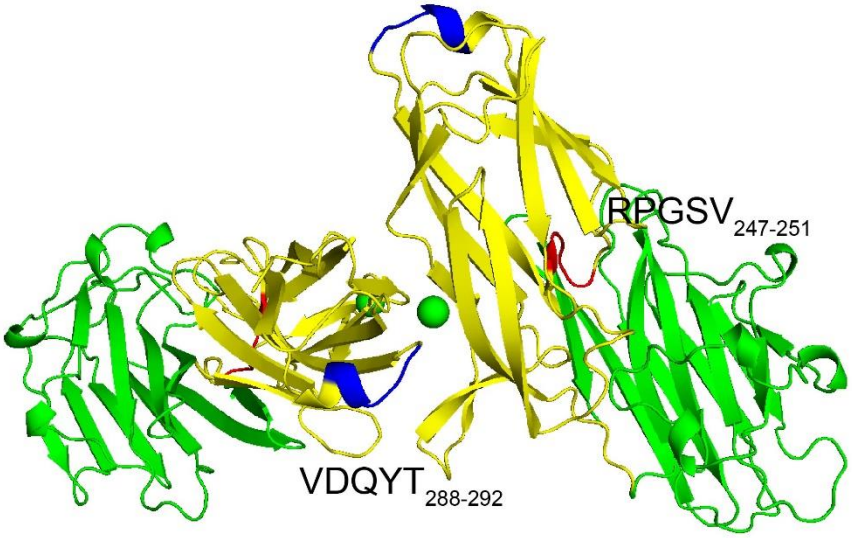

Figure S4. The structure of Ca^{2+}-bound SdrC protein. The $RPGSV_{247-251}$ and $VDQYT_{288-292}$ sequences are colored red and blue, respectively.

Table S1. Statistics of data collection and refinement

parameter	Sdrc-Ca^{2+}	SdrC (N2 interact with N3)
Data collection		
Wavelength (Å)	0.9785	0.9785
Beamline[a]	BL19U1	BL19U1
Space group	P212121	P212121
Cell dimensions		
a, b, c (Å)	48.485, 76.837, 79.625	61.753,120.333, 130.343,
α, β, γ (°)	90, 90, 90	90, 90, 90
Resolution (Å)	50.00-1.58	50.00-2.07
R_{merge} (%)	10.4 (71.7)	10.9 (47.4)
I/σI	39.4 (4.89)	36.3 (9.5)
Completeness (%)	99.9(100)	100(100)
Redundancy	12.9 (12.8)	13.3 (13.7)
Refinement		
Resolution (Å)	39.81-1.58	44.83-2.07
Reflections (#)	41307	59835
R_{work}/R_{free} (%)	16.68/20.38	17.46/22.57
Protein	2491	7354
Ca^{2+}	1	
Rmsds		
Bond lengths (Å)	0.023	0.018
Bond angle (°)	2.05	1.8
Average B factors ($Å^2$)		
Overall	18.99	30.62
Protein	17.24	30.08
Ca^{2+}	13.00	

Values in parentheses are for the highest-resolution shells. $R_{merge} = \sum(I-<I>)/\sum I$.
R-value = $\sum(|F_{obs}|-|F_{calc}|)/\sum|F_{obs}|$, where F_{calc} is the calculated protein structure factor from the atomic model (R_{free} was calculated with 5% of the reflections).

Table S2. primers and strains in this study

primers	5‘----3’
SdrC (178-496)-F	ATCTGGGTCTCACATGGCTCCACAACAAGGAACAAATGTTAATGATAAAG
SdrC (178-496)-R	ATCTGGGTCTCAAAGCTTATTTCTTTTGGTCGCCATTAGCAGTTGA
T7	TAATACGACTCACTATAGGG
SdrcN2(178-335)-F	ATCTGGGTCTCACATGGCTCCACAACAAGGAACAAATGTTAATGATAAAG
SdrcN2(178-335)-R	ATCTGGGTCTCAAAGCTTAATAATCGACAATGATTTCTTCGCTATATGTATCATTACCT
SdrCN2-D195AF	ATGTCCTTTATCAATCGCAATGGCAATATTTGAAAAATGTACTTTATCATTAACATTT
SdrCN2-D195AR	AAATGTTAATGATAAAGTACATTTTTCAAATATTGCCATTGCGATTGATAAAGGACAT
SdrCN2-D230AF	GTATCGCCCTCTTTAACAGAAGCATCGATTGTGTAATTTGCTT
SdrCN2-D230AR	AAGCAAATTACACAATCGATGCTTCTGTTAAAGAGGGCGATAC
SdrCN2-N293AF	GTTCAAAGCTACCTCTAACAGCTGTATATTGATCTACATAGTTCGTAAAAGTATATGTTGT
SdrCN2-N293AR	ACAACATATACTTTTACGAACTATGTAGATCAATATACAGCTGTTAGAGGTAGCTTTGAAC
SdrCN2-E329AF	GCTTAATAATCGACAATGATTTCTGCGCTATATGTATCATTACCTAAAG
SdrCN2-E329AR	CTTTAGGTAATGATACATATAGCGCAGAAATCATTGTCGATTATTAAGC
strains	E. coli DH5α
	E. coli BL21 (DE3)

Reference:

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