Biochemistry and Biophysics Reports 42 (2025) 101982

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Human neutrophil defensin-1 binding increases histidine kinase activity of SaeS in *Staphylococcus aureus*

Zhengfei Qi [a,b,d], Shuru Lin [c,d], Quanxiang Yu [d], Rui Ma [d], Kexin Zhang [d], Wenqi Jiang [d], Shurong Chen [a,b,d], Yilin Mai [d], Qingshan Bill Fu [a,b,c,d,*]

[a] *Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, 201203, China*
[b] *University of Chinese Academy of Sciences, Beijing, 100049, China*
[c] *College of Chinese Medicine, Guangzhou University of Chinese Medicine, Guangzhou, 510006, China*
[d] *Shanghai Institute of Materia Medica, Zhongshan Institute for Drug Discovery, Chinese Academy of Sciences, Zhongshan, 528400, China*

ARTICLE INFO

Keywords:
Two-component system
SaeS™
Bicelles
HNP1
Kinase activity

ABSTRACT

Human neutrophil defensin-1 (HNP-1) can specifically activate the SaeRS two-component system(TCS), which is essential for controlling virulence and immune evasion factors in *Staphylococcus aureus*. The reaction to HNP1 requires the transmembrane domain of SaeS (SaeS™), however the precise mechanism is yet unknown. In this work, we reconstructed the SaeS™ protein into bicelles and discovered that HNP1 can interact directly with SaeS™ using BiacoreT200, their binding significantly increases SaeS kinase activity and activated the SaeRS system subsequently. *Staphylococcus aureus* may exploit host-derived factors released by human immune cells to activate its two-component signal transduction system, thereby enhancing antimicrobial peptide resistance.

1. Introduction

Staphylococcus aureus is a Gram-positive pathogenic bacterium primarily colonizing the skin and mucous membranes [1]. While colonization itself is non-pathogenic, it constitutes a critical risk factor for progression to subsequent infections, which range from mild skin and soft tissue infections (SSTIs) to life-threatening invasive diseases including endocarditis, osteomyelitis, bacteremia, and fatal pneumonia [2]. Initially, penicillin demonstrated high efficacy in controlling *S. aureus* infections. However, the widespread clinical use of penicillin in the 1950s led to the emergence of penicillin-resistant *S. aureus* strains due to their ability to produce penicillinase—an enzyme that hydrolyzes the β-lactam ring, thereby conferring resistance [3,4]. The subsequent introduction of methicillin, which exhibits resistance to β-lactamase--mediated hydrolysis, temporarily controlled infections caused by penicillin-resistant *S. aureus* [5]. Notably, within two years, British scientist Jevons identified the first methicillin-resistant *S. aureus* (MRSA) strain, which rapidly evolved into a predominant multidrug-resistant pathogen across diverse global regions, including Europe, North America, North Africa, the Middle East, and East Asia [6,7]. Systemic analyses highlight that mortality attributable to MRSA antibiotic resistance represents a substantial clinical burden, rendering it a persistent challenge in both therapeutic management and biomedical research [8].

Staphylococcus aureus has evolved sophisticated regulatory networks to control the production of virulence factors, enabling bacterial adhesion to host tissues and immune evasion [9,10]. These networks include alternative sigma factors (e.g., σB), DNA-binding proteins (e.g., SarA and its homologs), and two-component regulatory systems (TCS; e.g., AgrAC, ArlRS, SrrAB, and SaeRS) [11–14], which adapt to temporal and spatial environmental changes. TCSs, composed of a sensor histidine kinase and a response regulator, represent a primary mechanism for bacterial perception of and response to environmental stimuli [15]. The *sae* operon (*S. aureus* exoprotein expression), encoding the SaeRS TCS, was first characterized by Giraudo et al., in 1994 through analysis of Tn551 mutants [16,17]. This operon comprises four genes: *saeP*, *saeQ*, *saeR*, and *saeS*. The histidine kinase SaeS detects specific environmental cues, with SaeP and SaeQ facilitating autophosphorylation at His131 via signal transduction. The phosphoryl group is subsequently transferred to Asp51 in SaeR, activating downstream signaling to drive expression of critical extracellular toxins and enzymes [18], including coagulase, α-toxin, β- and γ-hemolysins [16,19], Panton-Valentine leukocidin (LukGH) [20], toxic shock syndrome toxin-1 (TSST-1) [21], exfoliative toxins [22], and nucleases [15].These virulence and immune evasion factors impair host immune defenses. Studies have demonstrated that

* Corresponding author. Shanghai Institute of Materia Medica, Zhongshan Institute for Drug Discovery, Chinese Academy of Sciences, Zhongshan, 528400, China.
E-mail address: fuqingshan@simm.ac.cn (Q.B. Fu).

https://doi.org/10.1016/j.bbrep.2025.101982
Received 26 December 2024; Received in revised form 6 March 2025; Accepted 17 March 2025

S. aureus employs Sae-regulated staphylococcal nuclease (Nuc) and adenosine synthase A (AdsA) to neutralize the bactericidal activity of neutrophil extracellular traps (NETs). The enzymatic activity of these enzymes generates deoxyadenosine (dAdo), which triggers macrophage apoptosis [23], thereby indirectly disrupting the collaborative neutrophil-macrophage efferocytosis critical for NET formation. Additionally, Sae-regulated toxins (LukAB and LukED) damage phagocytes such as macrophages and neutrophils, enabling *S. aureus* to evade phagocytic killing [24]. These mechanisms collectively contribute to the development of antibiotic resistance.

The sensor histidine kinase SaeS is activated upon recognition of homologous signals such as high salt concentration, acidic pH [25], and human neutrophil defensins (HNPs) [26]. HNPs, a class of small antimicrobial peptides produced by neutrophils, are stored in azurophilic granules and released during infection or inflammation. These peptides exhibit antimicrobial, antiviral, and immunomodulatory functions, playing a pivotal role in microbial killing and degradation by phagocytes [27]. Geiger et al. [26] demonstrated that subinhibitory concentrations of HNP1-3 (0.5–20 μg/mL; MIC = 200 μg/mL) specifically activate the SaeRS two-component system, though the mechanistic basis for this specificity remains unresolved. Notably, Liu et al. [28] identified the transmembrane domain (TMD) of SaeS as both necessary and sufficient for responding to HNP-1, while also modulating the basal kinase activity of SaeS. Structural studies show that the TMD comprises two transmembrane helices flanking an extracellular loop of nine amino acids [29]. Mutagenesis analyses revealed critical functional insights: Schafer et al. [30] reported that the L18P mutation in the first transmembrane helix of Newman strain SaeS markedly enhances kinase activity, while Flack et al. [10] observed that alanine substitutions in the extracellular loop (particularly at conserved residues Met, Trp, and Phe) alter both basal activity and HNP1 responsiveness. Further, Liu et al. [28] demonstrated divergent effects of specific substitutions: M31A reduced kinase activity, whereas M31C increased it; similarly, F33Y suppressed activity, but F33V potentiated it. These findings underscore the TMD's central role in SaeRS activation, yet the precise activation mechanism, the molecular basis of HNP1 specificity, and whether HNP1 directly interacts with SaeS remain unknown.

Therefore, in this study, we expressed and purified the transmembrane domain of SaeS, reconstituted it into membrane-mimetic bicelles, and investigated its interaction with HNP1 *in vitro* using surface plasmon resonance (SPR). Concurrently, alterations in SaeS kinase activity were measured to assess the impact of HNP1 binding. Our findings demonstrate that HNP1 directly binds to the transmembrane domain of SaeS, significantly enhancing its kinase activity and thereby activating the SaeRS two-component system.

2. Materials and methods

2.1. Construction of Recombinant plasmids

The pMMLR6 plasmid and the designed SaeS™ sequence were sent to GenScript for completion of the SaeS™_pMMLR6 plasmid construction.BamHI/HindIII was the digestion site. Also, the designed $SaeS^{WT}$ sequence was sent to GenScript, using the pET22b(+) plasmid as the expression vector. NdeI/XhoI was used as the digestion site.

2.2. Expression and purification of SaeS™

The SaeS™ protein was overproduced in *E.coli* BL21 (DE3) harboring plasmid SaeS™_pMMLR6.Centrifuge overnight S. aureus cultures (OD600 = 2.0) at 10,000×*g* (4 °C, 15 min) to pelletize cells. Resuspend pellets in ice-cold lysis buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl) and disrupt via ultrasonication.Homogenize the lysate with 50 mL of guanidinium hydrochloride (GuHCl) buffer (6 M GuHCl, 150 mM NaCl, 50 mM Tris-HCl, 0.1 % Triton X-100, pH 8.0). Centrifuge at 15,000×*g* (30 min, 4 °C) to clarify; retain supernatant containing denatured SaeSTM. Incubate supernatant with 2 mL Ni-NTA resin and transfer slurry to gravity column, sequentially wash with 8 M urea buffer (8 M urea, 20 mM imidazole, 150 mM NaCl, 50 mM Tris-HCl pH 8.0), ddH_2O. The target protein SaeSTM was eluted with 12 mL of formic acid. React eluate with 1.5 g CNBr under slow nitrogen flow (amber vial, 1 h) and dialysis against ddH_2O (2 h, twice) using a 3.5 kDa MWCO membrane. Reconstitute lyophilized material in 0.5 mL hexafluoroisopropanol (HFIP)/0.5 mL H_2O/3 mL formic acid. Inject onto C18 column with gradient:Mobile Phase A: 5 % isopropanol, 95 % ddH_2O, 0.1 % TFA; Mobile Phase B: 75 % isopropanol, 25 % acetonitrile, 0.1 % TFA [38].

2.3. Reconstitution of SaeS™ into bicelles and transmission electron microscopy observation

The purified SaeS™ was recombined into bicelles formed of DMPC and DHPC, with a DMPC:DHPC ratio (q) of 0.5. 2 μL of sample was put in dropwise to the center of the copper mesh, followed by 2 μL of phosphotungstic acid dye solution after 1 min. The surplus dye solution was absorbed by filter paper. Repeated the dropwise dye solution procedure. Then prepared samples were delivered to the Sinoma Institute of Materials Research for transmission electron microscopy imaging.

2.4. Surface plasmon resonance (SPR) assay

SPR assay were performed on Biacore T200(GE Healthcare). HNP1 was purchased from MedChemExpress. According to the Easy Biacore SOP provided by Cytiva,freshly prepared SaeS™ bicelle(1 mg/mL) was diluted to 50 μg/mL with sodium acetate buffer (pH 4.0) and manually coupled on the CM5 chip to achieve the target amount. HNP1 was diluted with HEPES buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, pH 7.4) at gradient concentrations.The kinetic parameters were detected using a multi-cycle kinetic method, with the 120s contact time and the 300s dissociation time. The regeneration solution was glycine hydrochloride solution at pH 2.0, with a minimum of 5 cycles per experiment. Sensorgrams were analyzed using Biacore T200 evaluation software v3.0, and kinetic and affinity parameters (ka, kd and KD) were obtained by fitting the curve to a 1:1 binding model.

2.5. Expression and purification of $SaeS^{WT}$

The $SaeS^{WT}$ protein was overproduced in *E. coli* BL21 (DE3) carrying the plasmid SaeSWT_pET-22b(+). Overnight cultures were inoculated in 1 L LB fresh broth.When OD_{600} was close to 0.8, the proteins were expressed by the addition of 0.15 mM of isopropyl-1-thio-β-Dgalactopyranoside (IPTG) at 16 °C for 16 h. The bacteria were collected by centrifugation, resuspended in lysis buffer (50 mM Tris, 150 mM NaCl, 1 % NP40, pH 8.0), and the supernatant was collected after sonication. The supernatant was combined with a 2 mL Ni-NTA for overnight binding and eluted with buffer A (50 mM Tris, 150 mM NaCl, 0.05 % NP40, pH 8.0) containing different imidazole concentrations. Protein concentration was determined by dicinchoninic acid assay (Bio-Rad) and purified proteins were stored at −80 °C until used.

2.6. Kinase activity assay

Kinase activity of $SaeS^{WT}$ and the effect of HNP1 were assayed using the Kinase-Lumi™ Max Luminescent Kinase Assay Kit(Beyotime S0158). The reaction was set at room temperature in 50 μL of assay buffer (50 mM Tris, 150 mM NaCl, pH 8.0) containing $SaeS^{WT}$ (5 μM), HNP1 (10 μg/mL), and ATP (0–300 μM). Based on the instruction, The ATP standard curve was set up, 50 μL of detection Assay was added to the sample wells and standard wells and allowed to stay for 10 min. Luminescence Quick Read was performed with Glomax Navigator Microplate Detector (Promega) with a detection time of 0.5s.The data analysis was performed under the Michaelis-Menten kinetics by the Lineweaver-Burk

plot $\frac{1}{v} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$. The Km and V_{max} values were calculated using Lineweaver-Burk plot. The turnover number Kcat was calculated under this equation: $K_{cat} = \frac{V_{max}}{[E]}$.

3. Results

3.1. Purification of SaeS™

TrpLE is an anthranilate synthase fragment protein that facilitates the formation of inclusion bodies by sequestering hydrophobic proteins, which allows for high levels of protein expression.After cyanogen bromide cleaved the fusion protein with a methionine site, TrpLE and SaeS™ could be separated using reverse-phase HPLC. The chromatogram (Fig. 1A) showed the peaks of the purified SaeS™. The theoretical molecular weight of the SaeS™ is 6.7 kDa. As shown in lanes 2–4 of Fig. 1B, a band corresponding to 10–15 kDa was observed, indicative of the formation of a homodimer, consistent with prior literature reports.

3.2. SaeS™ reconstituted into bicelles and interaction with HNP1

Dilution of the SaeS™ bicelles with buffer containing 7 mM DHPC for maintenance of normal morphology. The bright discs in Fig. 2A represent the assembled SaeS™ bicelles, and the diameter of the bicelles noted by the wireframe is about 23 nm, which is in accordance with the theoretical value. Then, we coupled SaeS™ bicelles to the chip surface a small number of times in manual modern order to better replicate the binding and dissociation of SaeS™ with HNP1 in vivo.We performed a multi-cycle kinetic procedure by diluting the HNP1 analyte with HEPES buffer while trying to reduce the impact of solvent effects. A typical binding-sensing curve (Fig. 2B) was observed, which was fitted through a 1:1 binding model to estimate the constants of association (ka) and dissociation (kd) (Table 1). The KD of 1E-6 was obtained by $\frac{kd}{ka}$ calculations through experiments,which demonstrates that HNP1 has a direct and strong binding with SaeS™.

3.3. Purification of full length $SaeS^{WT}$

We inserted the full length $SaeS^{WT}$ sequence into the reading frame of the peIB signal peptide, which is included in the pET-22b(+) vector and can be expressed on the E.coli plasma membrane (Fig. S1A). Moreover, we supplemented the whole purification system with the descaling agent NP40 to promote hydrophobic proteolysis and maintain protein functions. $SaeS^{WT}$ was successfully purified, as shown by the 12.5 % SDS-PAGE result, and most of the protein remained in the supernatant after sonication (Fig. S1B). Buffer A containing 50 mM imidazole was used to elute $SaeS^{WT}$ from the Ni-NTA column.

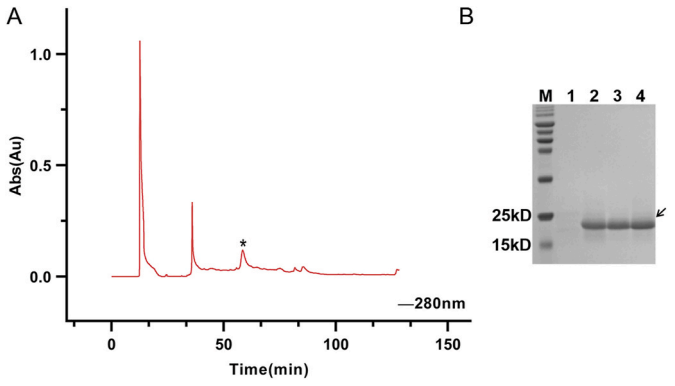

Fig. 1. Purification of SaeS™. a) HPLC chromatogram of cleaved TrpLE-SaeS™. b) Analysis of SaeS™ eluted peak by SDS-PAGE. M: Prestained protein ladder, 1: The upper band represents TrpLE-SaeS™ fusion protein and the lower band is TrpLE, 2–4: Purified SaeS™.

3.4. HNP1 can enhance $SaeS^{WT}$ kinase activity

According to the Kinase Assay Kit, kinase activity and ATP remaining are inversely correlated:the higher the kinase activity, the lower the luminescence value.The luminescence of the HNP1 group was noticeably less than that of the control group with various substrate concentrations (Fig. 3A). The residual ATP and the enzyme reaction rate were calculated based on the ATP standard curve (Fig. S2),the enzyme reaction rate of the HNP1 has been greatly increased(Fig. 3B). The Michaelis-Menten fitting (Fig. 3C) and the Lineweaver-Burk plot (Fig. 3D) were then executed. HNP1 can clearly raise kinase response rate while also improving V_{max} and conversion number (Table 2). Furthermore, HNP1 changed Km of $SaeS^{WT}$, which implies that HNP1 binding directly transforms its structure.

4. Discussion

Distinct from canonical sensor histidine kinases, SaeS represents a unique class of intramembrane-sensing kinases embedded predominantly within the lipid bilayer and is lack of surface receptors,which poses challenges for elucidating its structure and interaction mechanisms.To circumvent these limitations, we incorporated the SaeS TM into the plasmid fused with the TrpLE, achieving high-yield expression in inclusion bodies. Subsequent refolding was accomplished through the reconstitution of denatured membrane protein into bicelles by optimizing the mass ratio of DMPC and DHPC.In contrast to previous studies that employed MBP-tagged SaeS fusion proteins for purification [28,29], our current bicellar system facilitated the structural regeneration of the transmembrane domain into its native conformation. We quantitatively characterized the direct molecular interaction between SaeS TM and HNP-1 *in vitro* on BiacoreT200, resolving real-time kinetic parameters KD values despite negative results from prior intracellular yeast two-hybrid (Y2H) assays [28]. And HNP1 binding induced large increases in SaeS autokinase activity, manifested by elevated maximal reaction velocity (ΔVmax >124.8 %, $p < 0.01$) and turnover number (Δkcat >129.7 %, $p < 0.01$), accompanied by an increased Michaelis-Menten constant (Km). The change in the Km value also reflects a possible structural transformation in the catalytic centre of SaeS. (e.g., H-box/N-box/G-box motifs).

Our research shows that HNP-1 binds directly to SaeS and significantly enhances its autokinase activity. Then, the activation triggers the SaeRS to induce bacterial stress responses and global transcriptomic reprogramming. It upregulate the production of immune evasion factors (e.g., TNF-α, IFN-γ, IL-6, IL-2) [31,32] and neutrophil-targeted virulence determinants mentioned above [20], thereby modulating innate immune evasion of *S. aureus*. HNP1-driven adaptations recalibrate bacterial susceptibility to antibiotics and promote its antibiotic resistance.In addition, The Sae-regulon includes both the factors promoting biofilm formation (i.e., Coa, Emp, Eap, FnBPA, FnBPB, Hla, Hlb) [33,34]and biofilm dispersal factors (nuclease and proteases). Perturbations in SaeS kinase activity skew the transcriptional outputs toward elevated biofilm matrix synthesis and conferring resistance to both antimicrobial therapies and host defenses. Dubrac et al. [35] also identified WalKR as a transcriptional activator of nine genes (*lytM*, *atlA*, *isaA*, *sceD*, *ssaA*, and four *ssaA*-homologous genes*) that collectively mediate sequential stages of *Staphylococcus aureus* cell wall autolysis. Importantly, WalKR operon expression exhibits concentration-dependent co-variation with SaeRS activity [36]. Although the mechanism is not understood, SaeRS may also affect cell wall formation to influence bacterial susceptibility to antibiotics. Overall,HNP1-mediated SaeS agonism reveals an evolutionary trade-off: while enhancing bacterial immune evasion (via SaeRS virulence upregulation), it concurrently sensitizes *S. aureus* to TCS-targeted therapies [37]. Exploiting this vulnerability through antimicrobial peptides (AMP) and kinase inhibitors combinatorial regimens could counteract resistance evolution in chronic infections.

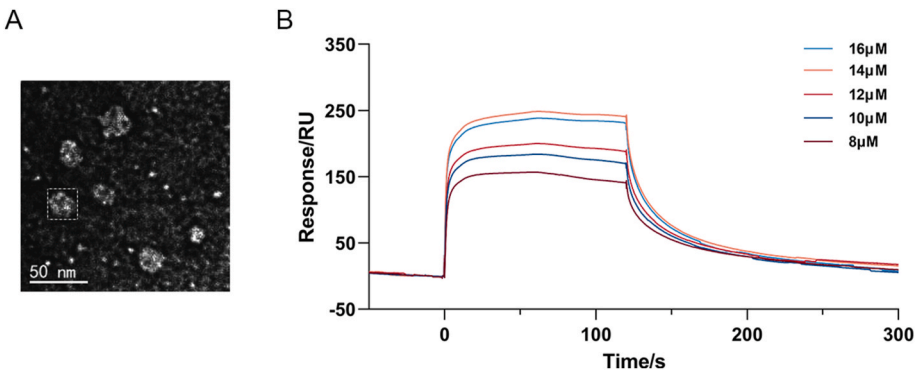

Fig. 2. Interaction curves of SaeS™ with HNP1. a) Transmission electron microscopy morphology image of reconstituted bicelles. b) Real-time sensing curve of the binding-dissociation of SaeS™ with HNP1.

Table 1
Kinetic parameters of HNP1 and SaeS™ interaction.

ka(1/Ms)	kd(1/s)	KD(M)
1.74E+4	1.86E-2	1.07E-6

CRediT authorship contribution statement

Zhengfei Qi: Writing – original draft, Investigation, Data curation. **Shuru Lin:** Visualization, Validation. **Quanxiang Yu:** Visualization, Validation. **Rui Ma:** Visualization, Validation. **Kexin Zhang:** Visualization, Validation. **Wenqi Jiang:** Visualization, Validation. **Shurong Chen:** Visualization, Validation. **Yilin Mai:** Visualization, Validation. **Qingshan Bill Fu:** Writing – review & editing, Supervision, Funding acquisition.

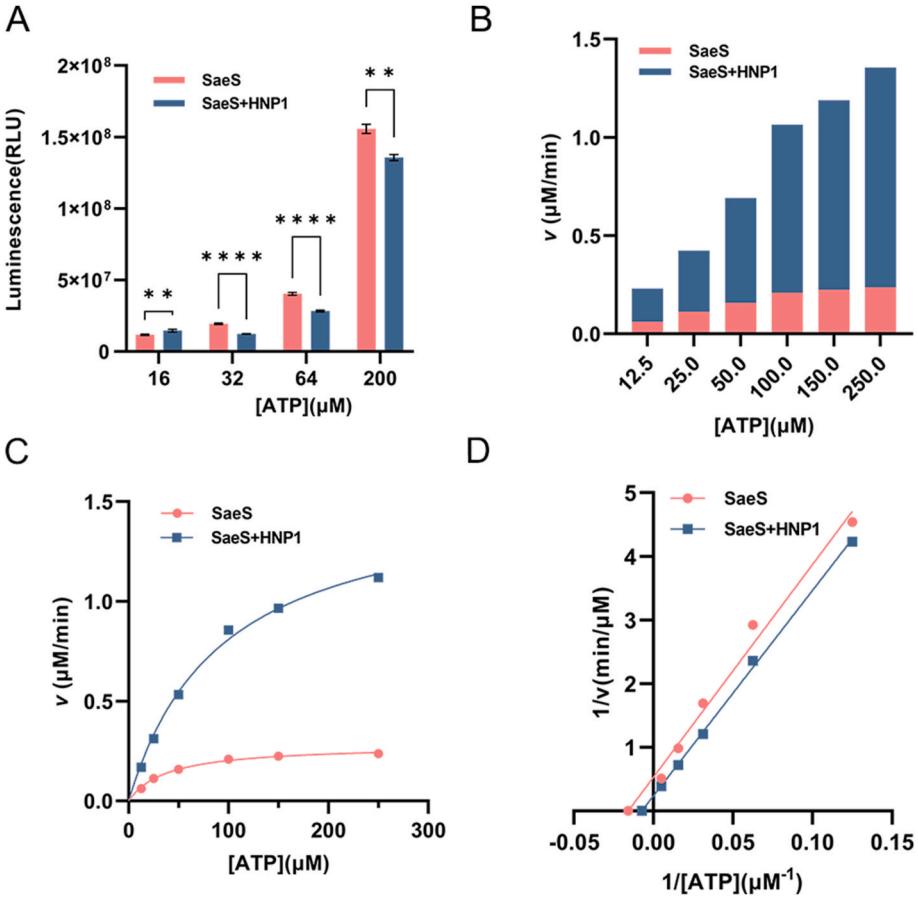

Fig. 3. Kinase activity assay. a) Comparison of fluorescence values with the addition of HNP1.Data are shown as Mean ± SD with three independent experiments.****,$p < 0.0001$. b) ATP standard curve: ATP concentrations were 0、1、2、4、8、16、32、64、128、200 μM. $Y = 1108070*X + 4182087$.$R^2 = 0.9952$. c) Michaelis-Menten fitting for the kinetic data of two groups:SaeS/SaeS + HNP1.The concentrations were 5 μM for SaeSWT and 10 μg/mL for HNP1. ATP concentrations were 0、1、2、4、8、16、32、64、128、200、250 μM. $R^2_{SaeS} = 0.9882$; $R^2_{SaeS + HNP1} = 0.9959$. d)Lineweaver-Burk plot for the kinetic data of two groups:SaeS/SaeS + HNP1.$Y_{SaeS} = 33.42*X + 0.5297$,$R^2 = 0.9836$; $Y_{SaeS + HNP1} = 32.29*X + 0.2356$,$R^2 = 0.9985$.

Table 2
Enzyme kinetic parameters of SaeS.

	Km (μM)	V_{max}(μM/min)	K_{cat} (min^{-1})
SaeS	63.09	1.8878	0.37
SaeS + HNP1	137.08	4.2445	0.85

Funding information

National Natural Science Foundation of China:32271320; Creative Research Group of Zhongshan City (Lingnan Pharmaceutical Research and Innovation team CXTD2022011).

Declaration of competing interest

We declared that There is No conflict of interest for this paper.

Acknowledgement

This work was supported by National Natural Science Foundation of China (Grants 32271320) and Creative Research Group of Zhongshan City (Lingnan Pharmaceutical Research and Innovation team CXTD2022011).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2025.101982.

References

[1] H.F. Wertheim, D.C. Melles, M.C. Vos, et al., The role of nasal carriage in Staphylococcus aureus infections, Lancet Infect. Dis. 5 (12) (Dec 2005) 751–762, https://doi.org/10.1016/S1473-3099(05)70295-4.
[2] F.D. Lowy, Staphylococcus aureus infections, N. Engl. J. Med. 339 (8) (Aug 20 1998) 520–532, https://doi.org/10.1056/NEJM199808203390806.
[3] C. Rayner, W.J. Munckhof, Antibiotics currently used in the treatment of infections caused by Staphylococcus aureus, Intern. Med. J. 35 (Suppl 2) (Dec 2005) S3–S16, https://doi.org/10.1111/j.1444-0903.2005.00976.x.
[4] S. Pichereau, W.E. Rose, Invasive community-associated MRSA infections: epidemiology and antimicrobial management, Expet Opin. Pharmacother. 11 (18) (Dec 2010) 3009–3025, https://doi.org/10.1517/14656566.2010.511614.
[5] E. Jokinen, J. Laine, R. Huttunen, et al., Comparison of outcome and clinical characteristics of bacteremia caused by methicillin-resistant, penicillin-resistant and penicillin-susceptible Staphylococcus aureus strains, Inf. Disp. 49 (7) (Jul 2017) 493–500, https://doi.org/10.1080/23744235.2017.1292046.
[6] J.R. Mediavilla, L. Chen, B. Mathema, B.N. Kreiswirth, Global epidemiology of community-associated methicillin resistant Staphylococcus aureus (CA-MRSA), Curr. Opin. Microbiol. 15 (5) (Oct 2012) 588–595, https://doi.org/10.1016/j.mib.2012.08.003.
[7] S. Lakhundi, K. Zhang, Methicillin-resistant Staphylococcus aureus: molecular characterization, evolution, and epidemiology, Clin. Microbiol. Rev. 31 (4) (Oct 2018), https://doi.org/10.1128/CMR.00020-18.
[8] C. Antimicrobial Resistance, Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis, Lancet 399 (10325) (Feb 12 2022) 629–655, https://doi.org/10.1016/S0140-6736(21)02724-0.
[9] Y.Q. Xiong, J. Willard, M.R. Yeaman, A.L. Cheung, A.S. Bayer, Regulation of Staphylococcus aureus alpha-toxin gene (hla) expression by agr, sarA, and sae in vitro and in experimental infective endocarditis, J. Infect. Dis. 194 (9) (Nov 1 2006) 1267–1275, https://doi.org/10.1086/508210.
[10] C.E. Flack, O.W. Zurek, D.D. Meishery, et al., Differential regulation of staphylococcal virulence by the sensor kinase SaeS in response to neutrophil-derived stimuli, Proc. Natl. Acad. Sci. U. S. A 111 (19) (May 13 2014) E2037–E2045, https://doi.org/10.1073/pnas.1322125111.
[11] X. Liang, L. Zheng, C. Landwehr, D. Lunsford, D. Holmes, Y. Ji, Global regulation of gene expression by ArlRS, a two-component signal transduction regulatory system of Staphylococcus aureus, J. Bacteriol. 187 (15) (Aug 2005) 5486–5492, https://doi.org/10.1128/JB.187.15.5486-5492.2005.
[12] A.L. Cheung, K.A. Nishina, M.P. Trotonda, S. Tamber, The SarA protein family of Staphylococcus aureus, Int. J. Biochem. Cell Biol. 40 (3) (2008) 355–361, https://doi.org/10.1016/j.biocel.2007.10.032.
[13] K.Y. Le, M. Otto, Quorum-sensing regulation in staphylococci-an overview, Front. Microbiol. 6 (2015) 1174, https://doi.org/10.3389/fmicb.2015.01174.
[14] C. Kong, H.M. Neoh, S. Nathan, Targeting Staphylococcus aureus toxins: a potential form of anti-virulence therapy, Toxins 8 (3) (Mar 15 2016), https://doi.org/10.3390/toxins8030072.
[15] C. Jenul, A.R. Horswill, Regulation of Staphylococcus aureus virulence, Microbiol. Spectr. 7 (2) (Apr 5 2019), https://doi.org/10.1128/microbiolspec.GPP3-0031-2018.
[16] A.T. Giraudo, C.G. Raspanti, A. Calzolari, R. Nagel, Characterization of a Tn551-mutant of Staphylococcus aureus defective in the production of several exoproteins, Can. J. Microbiol. 40 (8) (Aug 1994) 677–681, https://doi.org/10.1139/m94-107.
[17] A.T. Giraudo, A. Calzolari, A.A. Cataldi, C. Bogni, R. Nagel, The sae locus of Staphylococcus aureus encodes a two-component regulatory system, FEMS Microbiol. Lett. 177 (1) (Aug 1 1999) 15–22, https://doi.org/10.1111/j.1574-6968.1999.tb13707.x.
[18] Q. Liu, W.S. Yeo, T. Bae, The SaeRS two-component system of Staphylococcus aureus, Genes 7 (10) (Oct 3 2016), https://doi.org/10.3390/genes7100081.
[19] D.W. Jeong, H. Cho, H. Lee, et al., Identification of the P3 promoter and distinct roles of the two promoters of the SaeRS two-component system in Staphylococcus aureus, J. Bacteriol. 193 (18) (Sep 2011) 4672–4684, https://doi.org/10.1128/JB.00353-11.
[20] R.L. Watkins, K.B. Pallister, J.M. Voyich, The SaeR/S gene regulatory system induces a pro-inflammatory cytokine response during Staphylococcus aureus infection, PLoS One 6 (5) (2011) e19939, https://doi.org/10.1371/journal.pone.0019939.
[21] M.L. Baroja, C.A. Herfst, K.J. Kasper, et al., The SaeRS two-component system is a direct and dominant transcriptional activator of toxic shock syndrome toxin 1 in Staphylococcus aureus, J. Bacteriol. 198 (19) (Oct 1 2016) 2732–2742, https://doi.org/10.1128/JB.00425-16.
[22] F. Kato, N. Kadomoto, Y. Iwamoto, K. Bunai, H. Komatsuzawa, M. Sugai, Regulatory mechanism for exfoliative toxin production in Staphylococcus aureus, Infect. Immun. 79 (4) (Apr 2011) 1660–1670, https://doi.org/10.1128/IAI.00872-10.
[23] B.P. Howden, S.G. Giulieri, Fok Wong, T. Lung, et al., Staphylococcus aureus host interactions and adaptation, Nat. Rev. Microbiol. 21 (6) (Jun 2023) 380–395, https://doi.org/10.1038/s41579-023-00852-y.
[24] L. Munzenmayer, T. Geiger, E. Daiber, et al., Influence of Sae-regulated and Agr-regulated factors on the escape of Staphylococcus aureus from human macrophages, Cell. Microbiol. 18 (8) (Aug 2016) 1172–1183, https://doi.org/10.1111/cmi.12577.
[25] B. Weinrick, P.M. Dunman, F. McAleese, et al., Effect of mild acid on gene expression in Staphylococcus aureus, J. Bacteriol. 186 (24) (Dec 2004) 8407–8423, https://doi.org/10.1128/JB.186.24.8407-8423.2004.
[26] T. Geiger, C. Goerke, M. Mainiero, D. Kraus, C. Wolz, The virulence regulator Sae of Staphylococcus aureus: promoter activities and response to phagocytosis-related signals, J. Bacteriol. 190 (10) (May 2008) 3419–3428, https://doi.org/10.1128/JB.01927-07.
[27] K.A. Joiner, T. Ganz, J. Albert, D. Rotrosen, The opsonizing ligand on Salmonella typhimurium influences incorporation of specific, but not azurophil, granule constituents into neutrophil phagosomes, J. Cell Biol. 109 (6 Pt 1) (Dec 1989) 2771–2782, https://doi.org/10.1083/jcb.109.6.2771.
[28] Q. Liu, H. Cho, W.S. Yeo, T. Bae, The extracytoplasmic linker peptide of the sensor protein SaeS tunes the kinase activity required for staphylococcal virulence in response to host signals, PLoS Pathog. 11 (4) (Apr 2015) e1004799, https://doi.org/10.1371/journal.ppat.1004799.
[29] T. Mascher, Intramembrane-sensing histidine kinases: a new family of cell envelope stress sensors in Firmicutes bacteria, FEMS Microbiol. Lett. 264 (2) (Nov 2006) 133–144, https://doi.org/10.1111/j.1574-6968.2006.00444.x.
[30] D. Schafer, T.T. Lam, T. Geiger, et al., A point mutation in the sensor histidine kinase SaeS of Staphylococcus aureus strain Newman alters the response to biocide exposure, J. Bacteriol. 191 (23) (Dec 2009) 7306–7314, https://doi.org/10.1128/JB.00630-09.
[31] O.W. Zurek, T.K. Nygaard, R.L. Watkins, et al., The role of innate immunity in promoting SaeR/S-mediated virulence in Staphylococcus aureus, J. Innate Immun. 6 (1) (2014) 21–30, https://doi.org/10.1159/000351200.
[32] H. Cho, D.W. Jeong, Q. Liu, et al., Calprotectin increases the activity of the SaeRS two component system and murine mortality during Staphylococcus aureus infections, PLoS Pathog. 11 (7) (Jul 2015) e1005026, https://doi.org/10.1371/journal.ppat.1005026.
[33] M. Johnson, A. Cockayne, J.A. Morrissey, Iron-regulated biofilm formation in Staphylococcus aureus Newman requires ica and the secreted protein Emp, Infect. Immun. 76 (4) (Apr 2008) 1756–1765, https://doi.org/10.1128/IAI.01635-07.
[34] M. Zapotoczna, H. McCarthy, J.K. Rudkin, J.P. O'Gara, E. O'Neill, An essential role for coagulase in Staphylococcus aureus biofilm development reveals new therapeutic possibilities for device-related infections, J. Infect. Dis. 212 (12) (Dec 15 2015) 1883–1893, https://doi.org/10.1093/infdis/jiv319.
[35] S. Dubrac, I.G. Boneca, O. Poupel, T. Msadek, New insights into the WalK/WalR (YycG/YycF) essential signal transduction pathway reveal a major role in controlling cell wall metabolism and biofilm formation in Staphylococcus aureus, J. Bacteriol. 189 (22) (Nov 2007) 8257–8269, https://doi.org/10.1128/JB.00645-07.
[36] A. Delaune, S. Dubrac, C. Blanchet, et al., The WalKR system controls major staphylococcal virulence genes and is involved in triggering the host inflammatory

response, Infect. Immun. 80 (10) (Oct 2012) 3438–3453, https://doi.org/10.1128/IAI.00195-12.

[37] S. Tiwari, S.B. Jamal, S.S. Hassan, et al., Two-component signal transduction systems of pathogenic bacteria as targets for antimicrobial therapy: an overview, Front. Microbiol. 8 (2017) 1878, https://doi.org/10.3389/fmicb.2017.01878.

[38] Q. Fu, A. Piai, W. Chen, K. Xia, J.J. Chou, Structure determination protocol for transmembrane domain oligomers, Nat. Protoc. 14 (8) (Aug 2019) 2483–2520, https://doi.org/10.1038/s41596-019-0188-9.