Materials and Methods

Materials and reagents

SEA was kindly provided by Dr. Y.Q. Jiang from the Academy of Military Medical Sciences (Beijing, China). Ni-NTA spin columns were ordered from Roche (Indianapolis, IN). Plasmids, *E. coli* strains DH5α and BL21 (DE3) used for gene cloning and protein expression were bought from Novagen (Germany). Restriction enzymes, T4 DNA ligase, dNTP, and Pfu DNA polymerase were purchased from Takara (Japan). Anti-SEA polyclonal antibody was obtained from our previous study (Liang *et al.*, 2011, Chen *et al.*, 2014) and preserved in our laboratory previously. Anti-His-Tag HRP conjugated antibody and polyvinylidene difluoride (PVDF) membrane were bought from Bio-Rad (Mississauga, Canada).

Construction of pET-22b-scFv/p53 plasmid

The primers used for the construction of the expression vector were listed in Table S1. The scFv monomer gene (without p53) was constructed in a VH-linker-VL format together with a standard flexible 15 amino acid linker $(Gly_4Ser)_3$ in our previous work (Chen *et al.*, 2014). Briefly, total cell RNA was extracted from the hybridoma cell line 3C12 secreting anti-SEA monoclonal antibody (Liang *et al.*, 2011), which was then reversely transcribed into cDNA using RT-PCR. The cDNA was used as a template to amplify the light and heavy chain variable regions of the anti-SEA monoclonal antibody, which were then connected by a flexible peptide linker using SOE-PCR. Using the obtained anti-SEA scFv gene as a template, primers VR and VF were employed to amplify the target gene fragment containing the corresponding cleavage site.

The amplification of p53 tetrameric domain gene consists of 2 steps. Based on the sequence of p53 tetravalent functional domain gene and its IgG3 upstream hinge

region gene (Clore *et al.*, 1994), primers P1, P2, P3, P4 were designed and synthesized. Two rounds of overlap extension PCR was used to synthesize the p53 tetravalent functional domain gene using the P2 and P3 (94 °C 5 min; (94 °C 30 s, 55 °C 30 s, 72 °C 45 s) × 35 cycles; 72 °C 7 min). Then the PCR product was used as the template for the second step using the P1 and P4 (94 °C 5 min; (94 °C 30 s, 52 °C 30 s, 72 °C 30 s) × 35 cycles; 72 °C 7 min).

The human IgG upper hinge/human p53 tetramerization domain fusion gene was then fused to the N-terminal of anti-SEA scFv gene fragment. Then the fusion gene was digested with *Eco*R I and *Hin*d III followed by subcloning downstream of the His-Tag in the pET-22b expression vector (His6 tag; Novagen) to generate a pET-22b-scFv/p53 plasmid.

Expression and identification of the tetravalent scFv antibody

After successfully constructed, the pET-22b-scFv/p53 plasmid was then transformed into *E. coli* BL21 (DE3). The strain was cultured in 1 L of Luria-Bertani (LB) medium containing 100 μg/mL ampicillin at 37 °C with shaking at 200 rpm up to OD_{600} reaching 0.6-1.0. The cultures were induced with 1 mM IPTG and cultivated at 16 °C for 24 h. The cultures were obtained by centrifugation at 3300 × g for 5 min at 4 °C followed by washing and suspension in PBS buffer (50 mM KH_2PO_4, 300 mM NaCl, 5 mM EDTA, pH 8.0). Cells were lysed by sonication and debris was removed by centrifugation (8000 × g for 10 min at 4 °C), and the supernatant and pellet were analyzed by SDS-PAGE. Proteins resolved by SDS-PAGE were blotted on a polyvinylidene difluoride (PVDF) membrane (Bio-Rad, USA). The membrane was blocked with MPBS (5% skimmed milk in PBST) at 37 °C for 2 h and the protein was then detected by anti-His-Tag HRP conjugated antibody. The blot was developed using diaminobenzidine (DAB)-H_2O_2 solution.

Purification and concentration of the tetravalent scFv antibody

The crude extract from 1 L of *E. coli* culture was diluted and applied on 1 mL of pre-equilibrated Ni-NTA spin columns with 8 mL of binding buffer (50 mM KH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0). Then the Ni-NTA spin columns were washed with 12 mL of binding buffer. The target proteins were eluted with wash buffer (50 mM KH_2PO_4, 300 mM NaCl, 300 mM imidazole, pH 8.0). The protein concentration of purified tetravalent antibody was determined according to Bradford (Kruger, 2009) using BSA as a reference protein. To test whether the temperature and reducer affect the form of expressed target protein, the obtained tetravalent proteins were placed at 60 °C, 100 °C and room temperature for 10 min with 2 × sample loading buffer (unreduced and reduced) respectively. Then the proteins were identified by SDS-PAGE.

SEA-binding assay

The binding of tetravalent scFv to SEA was done by indirect ELISA (iELISA). ELISA plate wells were overnight coated with 100 μL of 1 μg/mL SEA in bicarbonate buffer at 4 °C. The coated wells were washed with PBST three times and then blocked with MPBS at 37 °C for 1 h. The plate was then incubated with different dilutions of the anti-SEA tetravalent antibody at 37 °C for 1 h. After three times washing, the bound tetravalent antibody was detected with anti-His-Tag HRP conjugated antibody. The reaction was stopped by adding 50 μL of 2 M H_2SO_4 and the optical density at 492 nm (OD_{492}) was recorded with an MK3 microplate reader (Thermo, USA).

Sandwich ELISA for SEA detection

Anti-SEA polyclonal antibody (pAb) was produced as previously described (Liang *et al.*, 2011) and used as the coating antibody and the tetravalent antibody was used as

detecting antibody. For pAb production, SEA was used as the immunization antigen (10 μg per animal). Multi-point back immunization was initially conducted, and after the first immunization, animals were injected with antigens intraperitoneally. Blood was collected and placed at 37 °C for 2 h for coagulation, then was centrifuged at 6000 r/min for 10 min. The supernatant was collected and stored at -20 °C until use.

ELISA plate wells were overnight coated with 100 μL of 4 μg/mL anti-SEA pAb at 4 °C. After three times washing with PBST, the wells were blocked with MPBS at 37 °C for 1 h. After three times washing with PBST, the wells were coated with 100 μL of SEA (0.25, 0.5, 1, 2, 4 μg/mL respectively) at 37 °C for 1 h. After three times washing with PBST, the coated wells were coated with 100 μL of the tetravalent antibody or scFv monomer for 1 h at 37 °C and then were washed with PBST. The wells were detected with the anti-His-Tag HRP conjugated antibody. The reaction was stopped by adding 50 μL of 2 M H_2SO_4 and the optical density at 492 nm (OD_{492}) was recorded with an MK3 microplate reader (Thermo, USA). For the determination of the effect of pAb concentration on the tetravalent antibody performance, ELISA plate wells were overnight coated with different concentrations of anti-SEA pAb and 100 μL of 20 μg/mL SEA was used as the target antigen. Other protocols were similar as above described.

Host information of SEA positive strains and scFv-SEA docking

The genome sequence of *S. aureus* strains was obtained from the National Center for Biotechnology Information (NCBI). The distribution of SEA in these strains was determined using BLASTN. Host information on SEA positive strains was obtained from NCBI. The structure of the scFv monomer and SEA was predicted and analyzed using the Phyre2 web portal (Kelley *et al.*, 2015). The scFv-SEA docking was performed using the online tool ClusPro 2.0 in antibody mode (Brenke *et al.*, 2012,

Kozakov *et al.*, 2017) and the result was visualized by the PyMOL molecular graphics system 2.0 (Schrödinger, LLC).

Statistical analysis

Data analysis was carried out in Prism 8.0 (GraphPad Software, La Jolla, CA, USA). The one way ANOVA with Tukey's multiple-comparison was applied to test the significance among groups at a significance level of $P < 0.05$.

References

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Table S1 Primers used for the construction of tetravalent scFv antibody

Primer	Sequence (5' to 3')
P1	CCG**CTCGAG**ACCCCACTTGGTGACACAACTCACACATCCGGAAAACCACTGGATGGAGAATAT
P2	GGGCGTGAGCGCTTCGAGATGTTCCGAGAGCTGAATGAGGCCTTGGAACTCAAG
P3	GGGCGTGAGCGCTTCGAGATGTTCCGAGAGCTGAATGAGGCCTTGGAACTCAAG
P4	CC**AAGCTT**TTAGTGGTGATGGTGATGATGTGGCTCCTTCCCAGCCTGGGCATCCTTGAGTTCCAAGGCCT
VR	CCG**CTCGAG**CCGTTTGATTTCCAGCTT
VF	CCG**GAATTC**CAGGTGCAGCTGCAGGAG