Supplementary Information

Nicking mechanism underlying the DNA phosphorothioate-sensing antiphage defense by SspE

Haiyan Gao¹,²#, Xinqi Gong³,#, Jinchuan Zhou¹, Yubing Zhang¹,², Jinsong Duan⁴, Yue Wei¹,⁵, Liuqing Chen², Zixin Deng¹, Jiawei Wang⁴, Shi Chen¹,⁵*, Geng Wu²*, Lianrong Wang¹*
Supplementary Table 1. Data collection and refinement statistics of SspE\textsubscript{CTD} from *S. scabiei* DSM 41658

|                      | Native SspE\textsubscript{CTD} | Se-Met SspE\textsubscript{CTD} |
|----------------------|---------------------------------|---------------------------------|
| **Data collection**  |                                 |                                 |
| Space group          | C222\textsubscript{1}           | C222\textsubscript{1}           |
| Cell dimensions      |                                 |                                 |
| \(a, b, c\) (Å)      | 108.7, 278.9, 182.3             | 107.9, 277.7, 181.8             |
| \(\alpha, \beta, \gamma\) (°) | 90, 90, 90                  | 90, 90, 90                   |
| Wavelength           | 0.97849 Å                      | 0.97893 Å                      |
| Resolution (Å)       | 50.00-2.73 (2.78-2.73)          | 50.00-3.00 (3.05-3.00)          |
| \(R_{merge}\)        | 0.116 (0.738)                  | 0.158 (1.189)                  |
| CC1/2                | 0.991 (0.912)                  | 0.994 (0.804)                  |
| \(I/\sigma_I\)       | 26.00 (2.071)                  | 16.00 (1.875)                  |
| Completeness (%)     | 99.8 (97.7)                    | 100.0 (100.0)                  |
| Redundancy           | 13.1 (11.2)                    | 13.2 (12.1)                    |
| **Refinement**       |                                 |                                 |
| Resolution (Å)       | 139.46-2.72                    |                                 |
| No. reflections      | 70397                           |                                 |
| \(R_{work}/R_{free}\) | 21.22%/26.50%                 |                                 |
| No. atoms            |                                 |                                 |
| Protein              | 14150                           |                                 |
| Water                | 0                               |                                 |
| B-factors            |                                 |                                 |
| Protein              | 81.246                          |                                 |
| R.m.s. deviations    |                                 |                                 |
| Bond lengths (Å)     | 0.0083                          |                                 |
| Bond angles (°)      | 1.3404                          |                                 |
| Ramachandran plot    |                                 |                                 |
| statistics (%)       |                                 |                                 |
| Most favorable       | 95.5                            |                                 |
| Additionally allowed | 4.0                             |                                 |
| Disallowed           | 0.5                             |                                 |

Only one crystal was used to identify the structure of SspE\textsubscript{CTD} from *S. scabiei* DSM 41658. Values in parentheses are for the highest-resolution shell.
**Supplementary Table 2. Data collection and refinement statistics of SspE from S. yokosukanensis DSM 40224**

|                      | Full-length SspE |
|----------------------|------------------|
| **Data collection**  |                  |
| Space group          | $P2_12_12_1$     |
| Cell dimensions      |                  |
| $a$, $b$, $c$ (Å)    | 109.28, 137.82, 292.32 |
| $\alpha$, $\beta$, $\gamma$ (°) | 90, 90, 90 |
| Wavelength           | 0.97849 Å        |
| Resolution (Å)       | 50.00-3.30 (3.42-3.30) |
| $R_{merge}$          | 0.259 (1.089)    |
| $CC1/2$              | 0.719 (0.779)    |
| $I/\sigma I$         | 12.4 (1.9)       |
| Completeness (%)     | 91.4 (93.6)      |
| Redundancy           | 13.3 (13.6)      |
| **Refinement**       |                  |
| Resolution (Å)       | 46.14-3.42       |
| No. reflections      | 61769            |
| $R_{work}/R_{free}$  | 20.1%/27.6%      |
| No. atoms            |                  |
| Protein              | 24344            |
| Water                | 0                |
| $B$-factors          |                  |
| Protein              | 94.2             |
| Water                |                  |
| R.m.s. deviations    |                  |
| Bond lengths (Å)     | 0.014            |
| Bond angles (°)      | 1.707            |
| Ramachandran plot statistics (%) |          |
| Most favorable       | 89.5             |
| Additionally allowed | 9.7              |
| Disallowed           | 0.8              |

Only one crystal was used to identify the structure of SspE from *S. yokosukanensis* DSM 40224. Values in parentheses are for the highest-resolution shell.
### Supplementary Table 3. Strains, plasmids, and phages used in this study

| Strains and plasmids | Characteristics | Source or reference |
|----------------------|-----------------|---------------------|
| **Strains**          |                 |                     |
| S. yokosukanensis    | 5'-C_P5CS-3' modification, GenBank: LMWN01000000 | DSMZ             |
| DSM 40224            |                 |                     |
| S. scabiei DSM 41658 | 5'-C_P5CS-3' modification, GenBank: NZ_LBNJ01000000 | DSMZ             |
| E. coli BL21(DE3)    | F' _ompT gal dcm lon hsdS2(rB'mB')_λ(DE3 [lac/lacUV5-T7p07 _ind1 sam7 nin5]) [malB^+](λ^3) | Novagen          |
| S. lividans HXY6     | S. lividans 1326 derivative lacking _dnd_ and _ssp_ genes | 1                |
| **Phages**           |                 |                     |
| JXY1                 | _Podoviridae_, lytic, dsDNA | 2                |
| **Plasmids**         |                 |                     |
| pBluescript II SK(+) | Cloning vector, 3 kb, Amp' | 3                |
| pUC19                | Cloning vector, 2.7 kb, Amp' | TransGen Biotech |
| pSET152              | _E. coli-Streptomyces_ shuttle vector, _aac(3)IV_, ColEI, att_P231, ori T | 4                |
| pPT551               | pET28a derivative expressing the CTD of _SspE_ from _S. scabiei_ DSM 41658, expression vector | This work        |
| pPT552               | pET28a derivative expressing the NTD of _SspE_ from _S. yokosukanensis_ DSM 40224, expression vector | This work        |
| pPT553               | pET28a derivative expressing the CTD of _SspE_ from _S. yokosukanensis_ DSM 40224, expression vector | This work        |
| pPT555               | pET28a derivative expressing _SspE_{K40A} from _S. yokosukanensis_ DSM 40224, expression vector | This work        |
| pPT556               | pET28a derivative expressing _SspE_{Q31A} from _S. yokosukanensis_ DSM 40224, expression vector | This work        |
| pPT557               | pET28a derivative expressing _SspE_{Y30A} from _S. yokosukanensis_ DSM 40224, expression vector | This work        |
| pWHU3643             | pET28a derivative expressing _SspE_{R100A} from _S. yokosukanensis_ DSM 40224, expression vector | 2                |
| pPT558               | pET28a derivative expressing _SspE_{R404AR408A} from _S. yokosukanensis_ DSM 40224, expression vector | This work        |
| pWHU3261             | pET28a derivative expressing _SspE_{N667A} from _S. yokosukanensis_ DSM 40224, expression vector | 2                |
| pPT560               | pET28a derivative expressing _SspE_{N667A} from _S. yokosukanensis_ DSM 40224, expression vector | This work        |
| Expression Vector | Description |
|-------------------|-------------|
| pPT561            | pET28a derivative expressing SspE<sub>N676A</sub> from *S. yokosukanensis* DSM 40224, expression vector |
| pPT562            | pET28a derivative expressing CFP, expression vector |
| pPT563            | pET28a derivative expressing YFP-Ssp-CFP, expression vector |
| pPT564            | pET28a derivative expressing YFP-CFP, expression vector |
| pPT565            | pET28a derivative expressing CTD<sub>K395A/R526A</sub> from *S. yokosukanensis* DSM 40224, expression vector |
| pPT566            | pET28a derivative expressing CTD<sub>R512A</sub> from *S. yokosukanensis* DSM 40224, expression vector |
| pPT567            | pET28a derivative expressing CTD<sub>R404A/R408A</sub> from *S. yokosukanensis* DSM 40224, expression vector |
| pPT568            | pET28a derivative expressing CTD<sub>396-771aa</sub> from *S. yokosukanensis* DSM 40224, expression vector |
| pWHU3658          | pSET52 derivative with a 9.7-kb fragment carrying *sspABCDE* from *S. yokosukanensis* DSM 40224 |
| pPT581            | pWHU3658 derivative expressing SspABCDE<sub>K40A</sub> |
| pPT582            | pWHU3658 derivative expressing SspABCDE<sub>R404A/R408A</sub> |
| pPT583            | pWHU3658 derivative expressing SspABCDE<sub>N667A</sub> |
| pPT584            | pWHU3658 derivative expressing SspABCDE<sub>N676A</sub> |
| pPT585            | pWHU3658 derivative expressing SspABCDE<sub>Q31A</sub> |
| pPT586            | pWHU3658 derivative expressing SspABCDE<sub>Y30A</sub> |
| pPT590            | pSET152 derivative with a 2.3-kb fragment carrying *sspE* from *S. yokosukanensis* DSM 40224 |
| pPT591            | pPT590 derivative expressing SspE<sub>K40A</sub> |
| pPT592            | pPT590 derivative expressing SspE<sub>R404A/R408A</sub> |
| pPT593            | pPT590 derivative expressing SspE<sub>N667A</sub> |
| pPT594            | pPT590 derivative expressing SspE<sub>N676A</sub> |
| pPT595            | pPT590 derivative expressing SspE<sub>Q31A</sub> |
| pPT596            | pPT590 derivative expressing SspE<sub>Y30A</sub> |
| pPT597            | pPT590 derivative expressing SspE<sub>Y30A</sub> |
Supplementary Table 4. Data collection and refinement statistics of SspE<sub>R100A</sub> from *S. yokosukanensis* DSM 40224

| SspE<sub>R100A</sub> | 
|---|---|
| **Data collection** | 
| Space group | *P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>* |
| Cell dimensions | 
| *a, b, c* (Å) | 110.03, 137.97, 292.35 |
| *α, β, γ* (°) | 90, 90, 90 |
| Wavelength | 0.97849 Å |
| Resolution (Å) | 50.00-3.50 (3.56-3.50) |
| *R<sub>merge</sub>* | 0.306 (2.296) |
| CC<sub>1/2</sub> | 0.988 (0.583) |
| *I/σI* | 15.0 (2.0) |
| Completeness (%) | 99.7 (85.8) |
| Redundancy | 12.9 (11.5) |
| **Refinement** | 
| Resolution (Å) | 36.54-3.48 |
| No. reflections | 54087 |
| *R<sub>work</sub>/R<sub>free</sub>* | 25.7%/30.8% |
| No. atoms | 
| Protein | 24462 |
| Water | 0 |
| *B*-factors | 
| Protein | 49.7 |
| Water | 
| R.m.s. deviations | 
| Bond lengths (Å) | 0.016 |
| Bond angles (°) | 1.829 |
| Ramachandran plot statistics (%) | 
| Most favorable | 87.4 |
| Additionally allowed | 11.9 |
| Disallowed | 0.7 |

Only one crystal was used to identify the structure of SspE<sub>R100A</sub> from *S. yokosukanensis* DSM 40224. Values in parentheses are for the highest-resolution shell.
Supplementary Fig. 1. Crystal structures of SspE\textsubscript{CTD} from \textit{S. scabiei} DSM 41658. 

(a) Ribbon diagram of the four molecules, shown in green, yellow, magenta, and cyan, in one asymmetric unit of SspE\textsubscript{CTD}. (b) Rotation (180°) of the SspE\textsubscript{CTD} monomer along the x-axis to show the back view. Helices are shown in cyan, and sheets are shown in magenta. Source data are provided as a Source Data file.
Supplementary Fig. 2. EMSA analysis of the binding of Ssp\textsubscript{ENTD} with increasing concentrations to linearized pUC19 DNA with or without PT modification at 5\textasciiquotesquot;-C\textsubscript{PS}CA-3\textasciiquotesquot;. The binding interaction between SspE and pUC19 DNA was used as a reference. Source data are provided as a Source Data file.
Supplementary Fig. 3. DNA nicking assay of SspE and SspE<sub>K40A</sub> toward plasmid DNA <em>in vitro</em>. In this assay, supercoiled pUC19 and pGM1190 DNA with or without the PT modification were used as substrates. Three hundred nanograms of plasmid DNA were incubated with 0.2 or 2 µM protein at 28 °C in CutSmart buffer (New England Biolabs). At the indicated time points, the nicked DNA products were analyzed on 1% agarose gels. PT-modified pGM1190 and pUC19 were isolated from SspABCD-SspE-expressing <i>S. lividans</i> HXY6 and <i>E. coli</i> cells, respectively. Source data are provided as a Source Data file.
Supplementary Fig. 4. Assessment of the antiplasmid activity of SspE in *E. coli* 3234/A. (a) Wild-type SspABCD-SspE module-containing *E. coli* 3234/A and Ssp-lacking mutant ΔsspBCDE were used as host strains for transformation by equal amounts of PT-modified (PT⁺) or non-PT-modified (PT⁻) pUC19, respectively. (b) The results are presented as relative transformation efficiencies (ratios of PT⁻/PT⁺ plasmid) obtained by parallel transformation of PT⁻/PT⁺ plasmid DNA. Data and error bars represent the mean ± SD from four independent experiments. Statistical significance was calculated by unpaired two-sided Student t-tests; NS, not significant. Source data are provided as a Source Data file.

**Supplementary References**

1. Liang, J. *et al.* DNA modification by sulfur: analysis of the sequence recognition specificity surrounding the modification sites. *Nucleic Acids Res.* **35**, 2944-2954 (2007).
2 Xiong, X. et al. SspABCD–SspE is a phosphorothioation-sensing bacterial defence system with broad anti-phage activities. *Nat. Microbiol.* **5**, 917-928 (2020).

3 Alting-Mees, M. A. & Short, J. M. pBluescript II: gene mapping vectors. *Nucleic Acids Res.* **17**, 9494 (1989).

4 Bierman, M. et al. Plasmid cloning vectors for the conjugal transfer of DNA from Escherichia coli to Streptomyces spp. *Gene* **116**, 43-49 (1992).