**SUPPorting Protocols**

**Protocol S1. Protein production for biochemical/biophysical studies**

The hypothetical proteins SehA and SehB were cloned using the Gateway (Life technologies, Carlsbad, CA, USA) in the destination vector pETG20A. To improve protein expression and solubility, both proteins were fused to thioredoxin (TRX) at their N-terminus [1]. The TRX was His-tagged and followed by an intervening region containing the tobacco etch virus (TEV) protease cleavage sequence.

SehA and SehB were expressed in the *E. coli* T7 Express Iq pLysS strain (New England Biolabs). Cells were grown in Terrific Broth at 37°C until the optical density reached 0.6 and protein expression was induced with 1mM isopropyl-β-thio-galactopyranoside (IPTG) overnight at 17°C. Cultures were harvested by centrifugation at 5.000 g for 20 min and resuspended in lysis buffer containing, 50 mM Tris–HCl pH 8.0, 300 mM NaCl, 10 mM imidazole, 0.25 mg/ml lysozyme, 2 mM PMSF and a cocktail of protease inhibitors (Complete EDTA-free, Roche). The cell pellets were then frozen at −80°C. For purification, cells were thawed at room temperature and incubated for 20 min at 4°C with 20 µg/ml DNAse I supplemented with 20 mM MgSO4. Cells were disrupted by sonication and the lysate clarified by centrifugation at 20.000g for 30 min at 4°C.

Proteins were loaded independently on a Ni2+-affinity chromatography column (HisTrap column, GE Healthcare) and eluted in a buffer containing 50 mM Tris–HCl pH 8.0, 300 mM NaCl, and 250 mM imidazole. Cleavage of the thioredoxin tag was achieved by the addition of TEV protease. The cleaved proteins were then recovered in the flow-through of a second Ni2+-affinity chromatography.

**Protocol S2. Multiangle light scattering**

The molecular weight was calculated using analytical size-exclusion chromatography (SEC) on a high-performance liquid chromatography (HPLC) system (Waters) coupled with online multiangle laser light scattering, ultra-violet light absorbance and refractive index detectors (MALLS/UV/RI) (Wyatt Technology, Santa Barbara, CA, USA). SEC was performed on a Shodex® KW-804 column with 25 mM HEPES pH 7.3, 150 mM NaCl as the eluent. The molecular weight was calculated using ASTRA V software (Wyatt Technology) with a refractive index increment (dn/dc) of 0.185 ml/g for protein samples. In the ‘protein conjugate’ analysis of SehB-DNA complexes we used extinction coefficients, at 280 nm, of 1.18 ml/(mg×cm) and 30.71 ml/(mg×cm) for SehB and for the DNA, respectively. In this analysis, the refractive index increment of DNA was set to 0.166 ml/g.

**Protocol S3. Surface Plasmon Resonance**

A Biacore® X100 Surface Plasmon Resonance system was used to study the direct binding of SehB to its promoter region. Oligonucleotides for SPR analysis (5′- AATTTTGTTTTCAACTTGAAAACTGGATA -3′ biotinylated in 3’ and 5’ TATCCAGTTTTCAAGTTGAAAACAAAATT 3’) were annealed by slow cooling in a buffer containing 20 mM Tris–HCl (pH 8.0) and 150 mM NaCl. Capture was performed at 25°C by injecting the biotinylated DNA over a research grade streptavidin (SA) sensor chip in a running buffer containing 10 mM HEPES (pH 7.3), 150 mM NaCl, and 0.005% (v/v) Tween-20. The DNA was immobilized at a concentration of 10 µg/ml resulting in approximately 300 response units (RUs) of immobilization. Purified SehB was then injected at concentrations ranging from 62.5 nM to 1µM over the immobilized DNAs at 25°C at a flow rate of 20 µl/min. Dissociation constants (KD) were derived by non-linear curve fitting of the standard Langmuir binding isotherm using the signal at the equilibrium response level with the GraphPad Prism version 5.00 for Macintosh (GraphPad Software, La Jolla California USA) using a 1:1 binding model.

**SUPPorting TABLES**

**Table S1. *Salmonella* strains and plasmids used in this study**

|  |  |  |
| --- | --- | --- |
| **Name** | **Description** | **Reference** |
| **Strains** |
| 12023 | Wild-type | Laboratory stock |
| ∆*sehB*  | 12023 ∆*sehB* ::Kmr | This work |
| *∆sehA*  | 12023 ∆*sehA* ::Kmr | This work |
| *∆sehAB*  | 12023 ∆(*sehA* *sehB*)::Kmr | This work |
| *∆sehABsc* | 12023 ∆(*sehA* *sehB*)::FRT | This work |
| *∆sehD* | 12023 ∆s*ehD*::Kmr | This work |
| *∆sehC* | 12023 ∆s*ehC*::Kmr | This work |
| *∆sehCD* | 12023 ∆(s*ehC* s*ehD*)::Kmr | This work |
| *∆sehAB* ∆*sehCD* | 12023 ∆*sehCD*::Kmr ∆*sehAB*::Cmr | This work |
| *sehB::3XFLAGsc* | 12023 *sehB*::3XFLAG-FRT | This work |
| *sehA::3XFLAGsc* | 12023 *sehA*::3XFLAG-FRT | This work |
| *∆sehB* *∆sehD*  | 12023 ∆*sehB*::Kmr ∆*sehD*::Kmr | This work |
| *∆sifA* | 12023 ∆*sifA*::Kmr | [2] |
| *∆ssaV* | 12023 ∆*ssaV*::Kmr | [3] |
| **Plasmids** |
| pDESTTM17 | *E. coli* expression system with GatewayR Technology.T7 promoter, IPTG inducible; ColE1; Apr | Invitrogen |
| pDEST-STM1550 | pDESTTM-17derivative expressing STM1550 toxin from T7 promoter | This work |
| pDEST-STM2954.1n | pDESTTM-17derivative expressing STM2954.1n toxin from T7 promoter | This work |
| pDEST-STM3032.2N | pDESTTM-17derivative expressing STM3032.2N toxin from T7 promoter | This work |
| pDEST-STM3516 | pDESTTM-17derivative expressing STM3516 toxin from T7 promoter | This work |
| pDEST-STM3558 | pDESTTM-17derivative expressing STM3558 toxin from T7 promoter | This work |
| pDEST-STM3777 | pDESTTM-17derivative expressing STM3777 toxin from T7 promoter | This work |
| pDEST-STM4031 | pDESTTM-17derivative expressing STM4031 toxin (SehA) from T7 promoter | This work |
| pDEST-STM4032.2N | pDESTTM-17derivative expressing STM4032.2N toxin (SehC) from T7 promoter  | This work |
| pDEST-STM4450 | pDESTTM-17derivative expressing STM4450 toxin from T7 promoter | This work |
| pDEST-PSLT028 | pDESTTM-17derivative expressing PSLT028 toxin from T7 promoter | This work |
| pDEST-PSLT106 | pDESTTM-17derivative expressing PSLT106 toxin from T7 promoter | This work |
| pBAD-DEST49 | Expression system with GatewayR Technology. pBAD promoter, arabinose inducible; Apr | Invitrogen |
| pBAD-SehB | pBAD-DEST49 derivative expressing SehB antitoxin from pBAD promoter | This work |
| pBAD-SehD | pBAD-DEST49 derivative expressing SehD antitoxin from pBAD promoter | This work |
| pBAD-STM3559 | pBAD-DEST49 derivative expressing STM3559 antitoxin from pBAD promoter | This work |
| pBAD-PSLT127 | pBAD-DEST49 derivative expressing PSLT127 antitoxin from pBAD promoter | This work |
| pMPM-K6 | Expression system under arabinose promoter; p15A1; Kmr | [4] |
| pK6-SehB  | pMPM-K6 derivative expressing SehB antitoxin from arabinose promoter | This work |
| pK6-SehD | pMPM-K6 derivative expressing SehD antitoxin from arabinose promoter | This work |
| pMPM-K3 | Expression system; p15A1; Kmr | [4] |
| pK3-SehAB  | pMPM-K3 derivative for expression of SehAB from its natural promoter | This work |
| pGST-SehB  | pDEST15 derivative for expression of GST-SehB antitoxin from the *lac* promoter | This work |
| pFPV25 | GFP reporter fusion vector; Apr | [5] |
| *sehAB-gfp* | pFPV25 derivative containing the *sehAB* promoter region | This work |
| *sehCD-gfp* | pFPV25 derivative containing the *sehCD* promoter region | This work |
| pKD46 | Lambda Red recombinase system under the arabinose promoter | [6] |
| pKD3 | Plasmid containing the Cm cassette for Lambda Red recombination | [6] |
| pKD4 | Plasmid containing the Km cassette for lambda Red recombination | [6] |
| pCP20 | Yeast Flp recombinase gene; temperature sensible | [6] |

**Table S2. Oligonucleotides used in this study**

|  |  |  |
| --- | --- | --- |
| **Primer** | **Sequence** | **Target gene** |
| **For gene deletion** |
| STA1A-H1P1 | ctatcggggtaataaagaATGAGAACTCATCGTCAGATGGATTGTAGGCTGGAGCTGCTTCG | *sehB* |
| STA1A-H2P2 | gtagctggacatatccaactactcgatgaaggcatctgctggCATATGAATATCCTCCTTAG | *sehB* |
| STA1T-H1P1 | ttgtaatggaactacattGTGCATGTTATCAGCCGAAAACCGTGTAGGCTGGAGCTGCTTCG | *sehA* |
| STA1T-H2P2 | ccatctgacgatgagttctcattctttattaccccgatagtaCATATGAATATCCTCCTTAG | *sehA* |
| STA2T-H1P1 | taagccaatggcgtatggaatatgcaatttatagaaacggaatgtaggctggagctgcttcg | *sehC*  |
| STA2T-H2P2 | CTCAAATAACACTTTATCCATCTACCACCTCTCATTCAGCATCATATGAATATCCTCCTTAG | *sehC* |
| STA2A-H1P1 | ATGCTGAATGAGAGGTGGTAGATGGATAAAGTGTTATTTGAGTGTAGGCTGGAGCTGCTTCG | *sehD*  |
| STA2A-H2P2 | CTAACGTTAGCCGGATCATTGCTCAATAACGCAATGCTTGTATCATATGAATATCCTCCTTAG | *sehD* |
| **For *gfp* transcriptional fusion** |
| STA1T-EcoRI-5 | acggaattctcaggctacgagatgtatatgccgcgaacc | *sehAB* |
| STA1T-BamHI-3 | aatggatccccattacaaagttttcaacctgaaaac | *sehAB* |
| STA2T-EcoRI-5 | gcGAATTCtcatggcatcgagttc | *sehCD*  |
| STA2T-BamHI-3 | tcggatcctataaattgcatattcc | *sehCD*  |
| **For gene cloning** |
| STM1550-GTW-5 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACTTATAAGCTGGCATTT | STM1550 |
| STM1550-GTW-3 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAgcttctgtgtcgtgtcat | STM1550 |
| STM2954.1n-GTW-5 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGTAAAATTAACGCCAAAGGCCAGTG | STM2954.1n |
| STM2954.1n-GTW-3 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATAACCAATTTACATGCTTACCAGCATCCAG | STM2954.1n |
| STM3033-GTW-5 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCTGAAATTCATGCTTGATACCAAT | STM3033 |
| STM3033-GTW-3 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAgcACCAGTCTTCGATTCGGATACC | STM3033 |
| STM3516-GTW-5 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGGGCAAAGGGAAATTGAATATTCG | STM3516 |
| STM3516-GTW-3 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAAAATAAATCGGCATGCGTTCC | STM3516 |
| STM3558-GTW-5 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCCTACAACTTATCTCAGCGG | STM3558 |
| STM3558-GTW-3 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGGCACGTAAGCGAACGGCAATTTGC | STM3558 |
| STM3777-GTW-5 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCGAACCTTCAAAACCAGGTGGTTTAAC | STM3777 |
| STM3777-GTW-3 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGTTTTTGCTGACATGGCGCGCC | STM3777 |
| STM4031-GTW-5 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCATGTTATCAGCCGAAAAC | *sehA* |
| STM4031-GTW-3 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAttctttattaccccgatag | *sehA* |
| STM4032.2N-GTW-5 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCAATTTATAGAAACGGAA | *sehC* |
| STM4032.2N-GTW-3 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAccacctctcattcagcat | *sehC* |
| STM4450-GTW-5 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACTTATGAACTGGAATTC | STM4450 |
| STM4450-GTW-3 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAaagccgtttgtttgcatc | STM4450 |
| PSLT028-GTW-5 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCAGTTTAAGGTTTACACCTGTAAAAGG | PSLT028 |
| PSLT028-GTW-3 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAgATCCCCCGGAACATCAGGTTAATGGC | PSLT028 |
| PSLT106-GTW-5 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCTGAAGTTTATGCTGGATACTAAC | PSLT106 |
| PSLT106-GTW-3 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGCTCCAGTCCTCAGTTCTCAGTCC | PSLT106 |
| STM3559-GTW-5 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCTTTATGCGTACGGTTAACTATAGC | STM3559 |
| STM3559-GTW-3 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATTTATCCGCCAGCTCCCTGAGCTC | STM3559 |
| PSLT127-GTW-5 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCAAGCAGCGAATTACAGTGACAGTG | *PSLT127* |
| PSLT127-GTW-3 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACCAGTTCCTGTTGTCGTCAGC | *PSLT127* |
| STA1A-GTW-5 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCAgaactcatcgtcagatggatgc | *sehB* |
| STA1A-GTW-3 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTActcgatgaaggcatctgctgg | *sehB* |
| STA2A-GTW-5 | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCgATAAAGTGTTATTTGAG | *sehD* |
| STA2A-GTW-3 | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAACGCAATGCTTGTATAAC | *sehD* |
| STA1A-NcoI-5 | AAACCATGGGAACTCATCGTCAGATGCATGC | *sehB* |
| STA1A-HindIII-3 | TCGAAGCTTATGAAGAGGGCGGG | *sehB* |
| STA2A-NcoI-5 | TGGTCCATGGATAAAGTGTTATTTGAGCG | *sehD* |
| STA2A-HindIII-3 | CCAAGCTTGTTCCCGGGGGAAGGGG | *sehD* |

**SUPPorting REFERENCES**

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