Science Advances

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Supplementary Materials for

Crystal structure of a YeeE/YedE family protein engaged in thiosulfate uptake

Yoshiki Tanaka, Kunihito Yoshikaie, Azusa Takeuchi, Muneyoshi Ichikawa, Tomoyuki Mori, Sayaka Uchino, Yasunori Sugano, Toshio Hakoshima, Hiroshi Takagi, Gen Nonaka, Tomoya Tsukazaki*

*Corresponding author. Email: ttsukazaki@mac.com

The PDF file includes:

Figs. S1 to S5

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/35/eaba7637/DC1)

Movie S1

YeeE

Block 1 — helices H1, LA, H2, H3

```
                                   H1              LA        H2              H3
Spirochaeta_thermophila   ---MIWTGLLVGFLFGIVLQRGRICFNSAFRDVLLFKDNYLFKLAVFTLALEMILFVLLS   57
Dictyoglomus_thermophilum ---MIWQGLLVGILFGVVLQRSRMCFNSAIRDIKFNKDNYLVKMAAVAILIETLGFHLVA   57
Escherichia_coli          MFSMILSGLICGALLGFVMQRGRFCLTGGFRDMYIVKNNRMFYALLIAISVQSVGFFVLI   60
Basfia_succiniciproducens ----MLIGLFIGLLFGFFLQRGQFCFVSGFRIIYTQRNFRFLTALLIAVSIQSIGFFSLS   56
                             :  **: * *:*..:**.::*: ..:*  : ::   :.    .:: :: : * :
ConSurf Grade             96169715914896789979798989899944656857867763577466555564184
```

Block 2 — helices H4, LB, H5, H6

```
                                      H4          LB    H5              H6
Spirochaeta_thermophila   QVGLMQMNPKPLNLVGNIIGGFVFGLGMVLAGGCASGVTYRVGEGLTTAWFAALFYGLGA   117
Dictyoglomus_thermophilum SLGWIKLNPLPFIPLAQIIGGFLFGMGMVLAGGCASGVTYRIGEGYITAILAGLFYGITA   117
Escherichia_coli          QAGLLTYEAGAFPWLGTVIGGYIFGLGIVLAGGCATGTWYRAGEGLIGSWIALFYMVMS   120
Basfia_succiniciproducens GLDLITIPNTPMPLLATLIGGLLFGIGMVLANCCASGGWFRTGEGAVGSWIALICFALTM   116
                            . :       :  :. :*** :**:*:***. **:*   :* ***    : :* : :
ConSurf Grade             31817141211533287749745994998889999999889958999789319812755238
```

Block 3 — helix H7

```
                                                                 H7
Spirochaeta_thermophila   YATKSGAFSWWLSWVGQFKSPLSVEESAYY--VKGAGPTISSVLGLNPWIPALVIAALFI   175
Dictyoglomus_thermophilum SAVRGGVLNFVNSWFGSPITVTMQDPGIYNAVEGKVSPTIANVLGINPWIVAIVFAVLLA   177
Escherichia_coli          AVMRSPHASGLNQTLQHY--------------STEHNSIAETFNLSVWPLVAVLLVITL   165
Basfia_succiniciproducens AATQTGALKQWINPLLLE--------------TTTLDNIYNTFNLSPWILVTVLVLITV   161
                            . :      .   .  .               . *  ..:.:. *  .  *:  :
ConSurf Grade             367519182111112114355131111116   1111238633327374823613421151
```

Block 4 — helix H8, LC

```
                                                                 H8              LC
Spirochaeta_thermophila   LWA-FGTKTTSRE--------------T--KFNWKIASVCLALVAGLGFITSTLSGRKY   217
Dictyoglomus_thermophilum IYI-WGTKTTERQV-------------S--GLNWLTGGVALGIVGILGY----LSQKSY   216
Escherichia_coli          WVVMKELKKPKLKVATLPPRRTGIAHILFEKRWHPFVTAVLIGLIALLAWPLSEATGRMF   225
Basfia_succiniciproducens VMIVYHIKNPRYQFPQE-PTTALIPHRIFTKHWHPFTAAVWIGLLGVLAWLVSEQYGRSY   220
                             *.    :                      6    .*  :.::. *.:  :
ConSurf Grade             125 124644153                  6   4447217733682763386249156 8844
```

Block 5 — helices H9, H10

```
                                    H9                       H10
Spirochaeta_thermophila   GLGITGGWINLFQGFLTNS--PLNWEGLEIVGIILGAGVAAAVAGEFKLRMPKNPVTYLQ   275
Dictyoglomus_thermophilum ALGITGGWVNLLRGTVSGV--AYNWIGMEVLGIIIGAFVSALISKEFKLRVPKDPKTYLQ   274
Escherichia_coli          GLGITSPTANILQFLVAGDVKYINWGVFLVLGIFVGSFIAAKASREFRVAA-DAQTTLR   284
Basfia_succiniciproducens GYGVAVPTANVVQYIVIGQQRYLNWGSYFVLGILLGSFIAAKLSGEFEIRLP-EPKAILQ   279
                            . *::     *:.:   : .       **    :::**::*: ::*   : : *:
ConSurf Grade             7799978756611123451  1369265674986398268941559975969734175155
```

Block 6 — helices H11, LD, H12, H13

```
                            H11          LD    H12              H13
Spirochaeta_thermophila   VGIGGLLMGIGAVTAGGCNIGHFLTGVPQLALSSWLASIFFILGNWTMAWILFRR-----   330
Dictyoglomus_thermophilum VILGGIILMGFGAGVAAGCNIGHILSGLPHLALSSILATIFFVLGNWFMFWYLYARK----   330
Escherichia_coli          SGLGGVLMGFGASIAGGCSIGNGLVMTAMMTWQGWIGLVFMILGVWTASWLMYVRPQRKA   344
Basfia_succiniciproducens RMLGGVLMGIGASLAGGCTITNALVSTAYFSWQGWLATLMIMIGCWLTSVLVKPTQCRI-   338
                             :**::**:** *.**.* : *       :: .. :. :::::: *         :
ConSurf Grade             7419915993993338599999996895766775697376177669782876167
```

Block 7 — C-terminal

```
Spirochaeta_thermophila   -------- 330
Dictyoglomus_thermophilum -------- 330
Escherichia_coli          RLATAAAN 352
Basfia_succiniciproducens -------- 338
```
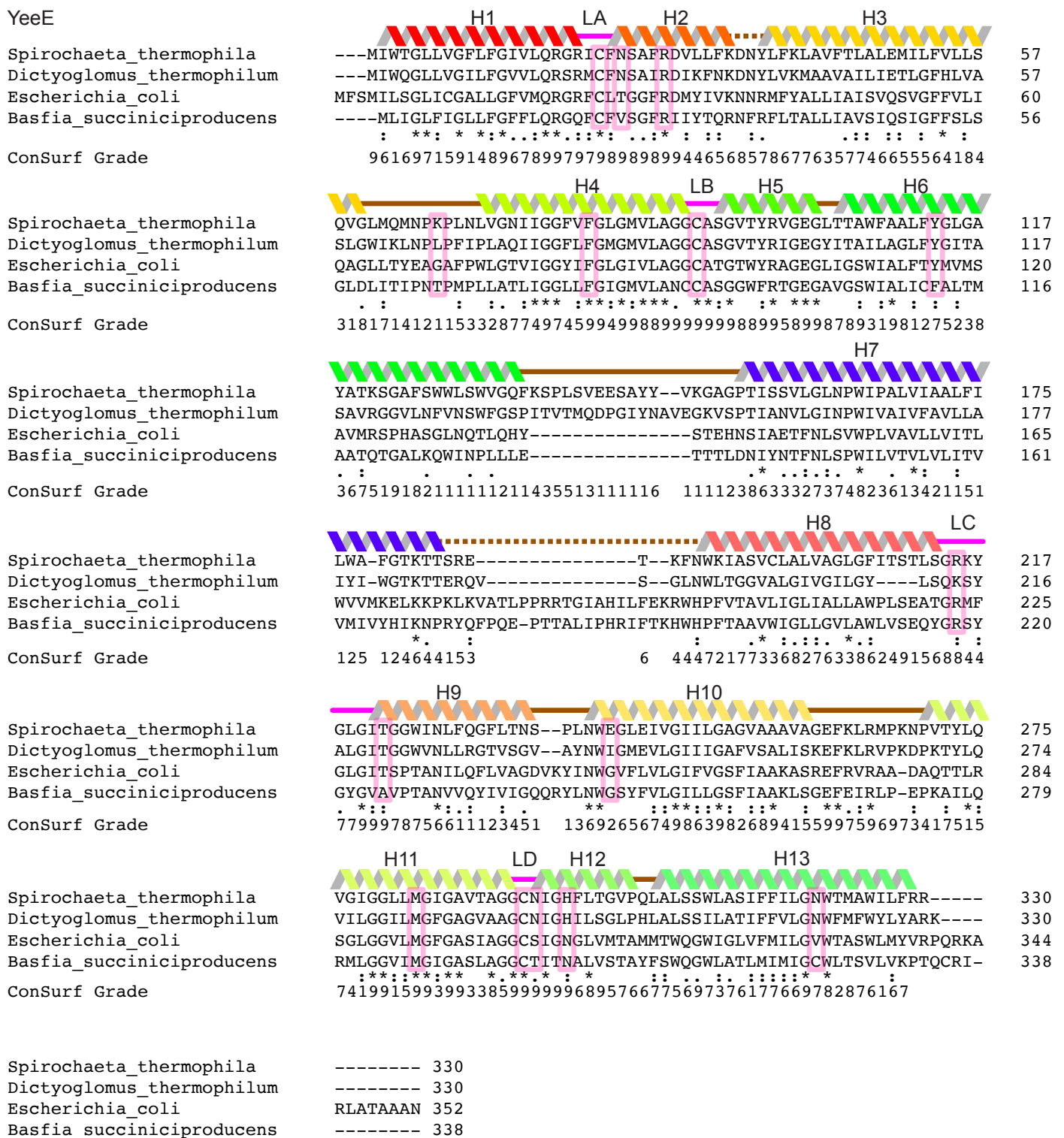
Fig. S1. Sequence alignment of YeeEs. Amino acid sequences of YeeEs from *Spirochaeta thermophila* (NCBI Reference Sequence: WP_014624389), *Dictyoglomus thermophilum* (WP_012548696), *Escherichia coli* (WP_072769027), and *Basfia succiniciproducens* (WP_100051949) were aligned by Clustal Omega (EMBL-EBI). The loops LA–LD and α-helices H1–H13 are indicated. The α-helices are colored as in Fig. 2. The mutated positions for *E.coli* YeeE functional analysis are indicated by magenta boxes. The amino acid sequence of *Spirochaeta thermophila* YeeE was analyzed by the ConSurf web server (https://consurf.tau.ac.il) with default settings. The resulting ConSurf conservation grades, derived from a multiple sequence alignment of ConSurf-selected 90 amino acid sequences, are shown.
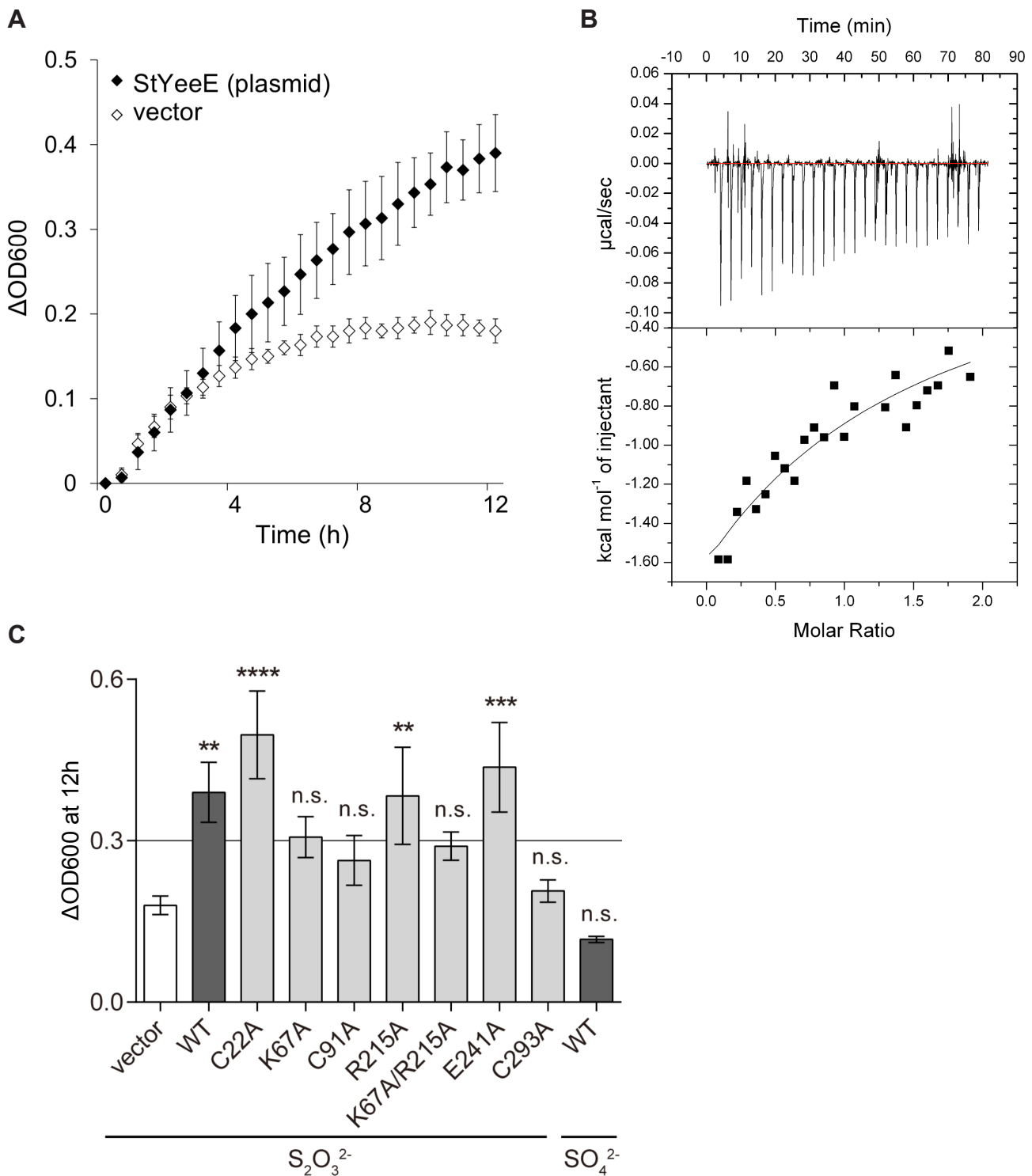
Fig. S2. Functional analyses of Spirochaeta thermophila YeeE (StYeeE). (**A**) Growth complementation of Δ*cysPUWA* Δ*yeeE* (DE3) in the single sulfur source minimal media containing thiosulfate by expression of StYeeE. OD600 was monitored every 30 min. Error bars indicate the standard deviation (n = 3). (**B**) ITC data for titration of StYeeE with thiosulfate ion. 500 μM sodium thiosulfate was titrated against 56.2 μM StYeeE. Top panel shows the raw heat of binding after subtracting the heat of dilution. Bottom panel represents the integrated heat. Nonlinear curve fitting with one set of sites model estimated that Kd, ΔH and ΔS values are 251 μM, -1.52 x 10^6 cal/mol and -5.15 x 10^3 cal/mol/ deg, respectively. (**C**) Growth (ΔOD600 at 12 h) of Δ*cysPUWA* Δ*yeeE* (DE3) expressing the indicated YeeE variants in the single sulfur source minimal media containing thiosulfate or sulfate. Error bars indicate the standard deviation (n = 3). Statistical significances compared with the vector were determined using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test (**p < 0.01; ***p < 0.001; ****p < 0.0001; n.s., not significant).
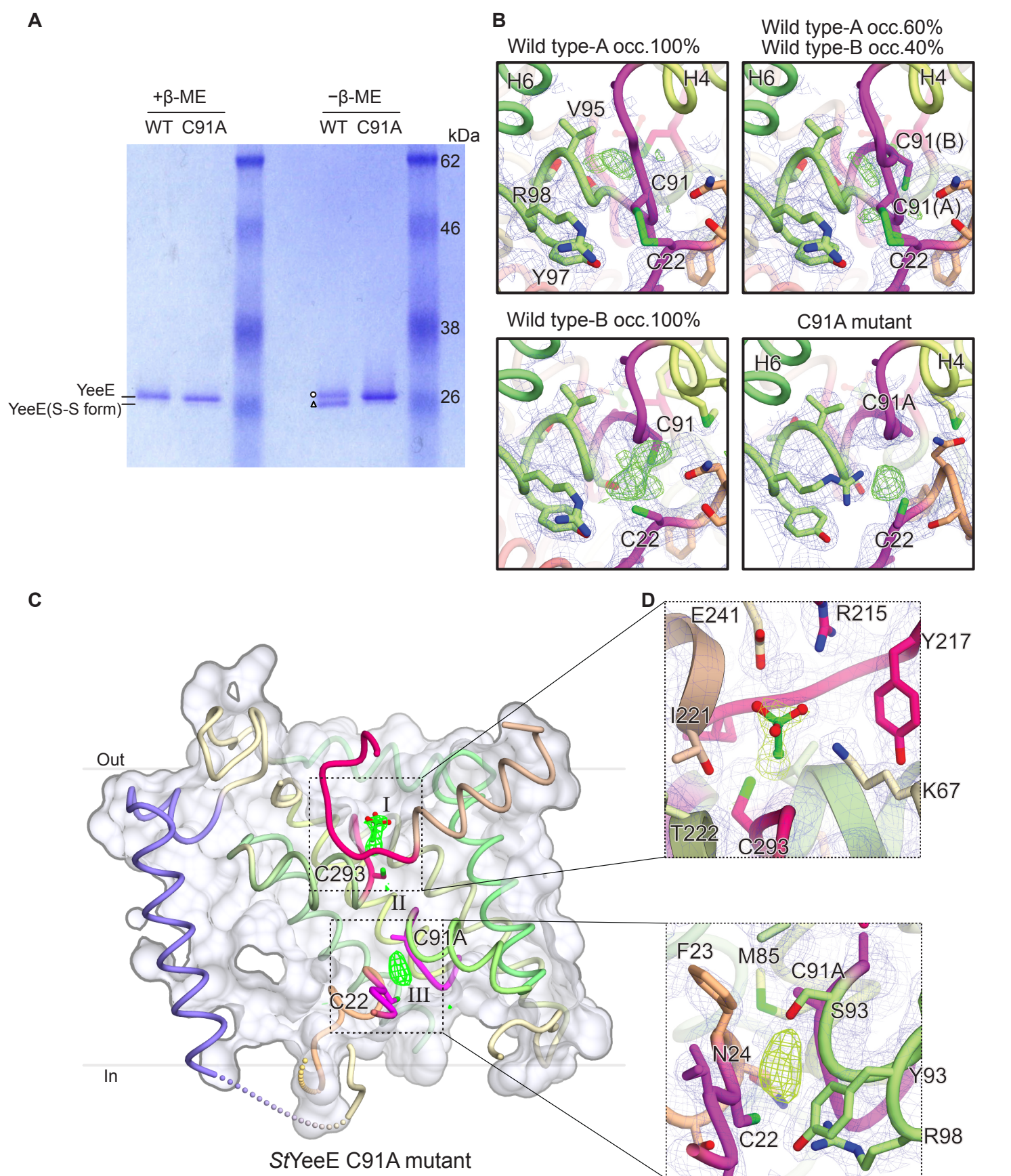
Fig. S3. Crystal structure of YeeE in reduced state. (**A**) Purified YeeE. Purified YeeE or C91A mutant was mixed with 2× SDS-PAGE sample buffer (125 mM Tris-HCl pH6.8, 4% SDS, 20% glycerol) supplemented with 5% β-ME or 5 mM 2-iodoacetamide and incubated for 30 min at room temperature, then analyzed by SDS-PAGE. This panel supports that some purified WT YeeE possesses a disulfide bond between C22 and C91. (**B**) Alternative conformations in WT crystal structure. Close-up views around the C22 residue of WT and C91A mutant. Each *Fo-Fc* map was calculated using the indicated occupancy for WT or using the C91A mutant structure and shown with 3.5 σ (green mesh). When the ratio of type-A to type-B was 3:2, the Fo-Fc map was smallest around the C22 residue. The 2*Fo-Fc* map with 1.0 σ is shown in blue. (**C**) Cross-sectional surface and tube models of YeeE C91A mutant. The thiosulfate-omit (*Fo-Fc*) map around positions I–III with 4.0 σ are shown as green mesh. The C22, A91, and C293 residues and thiosulfate are shown as a stick model. (**D**) Close-up views of around positions I (upper) and III (bottom) as enclosed in **C**. The 2*Fo-Fc* map with 1.2 σ and thiosulfate-omit (*Fo-Fc*) map with 4.0 σ are shown in blue and green mesh, respectively. The side chains and thiosulfate are emphasized in the stick model.
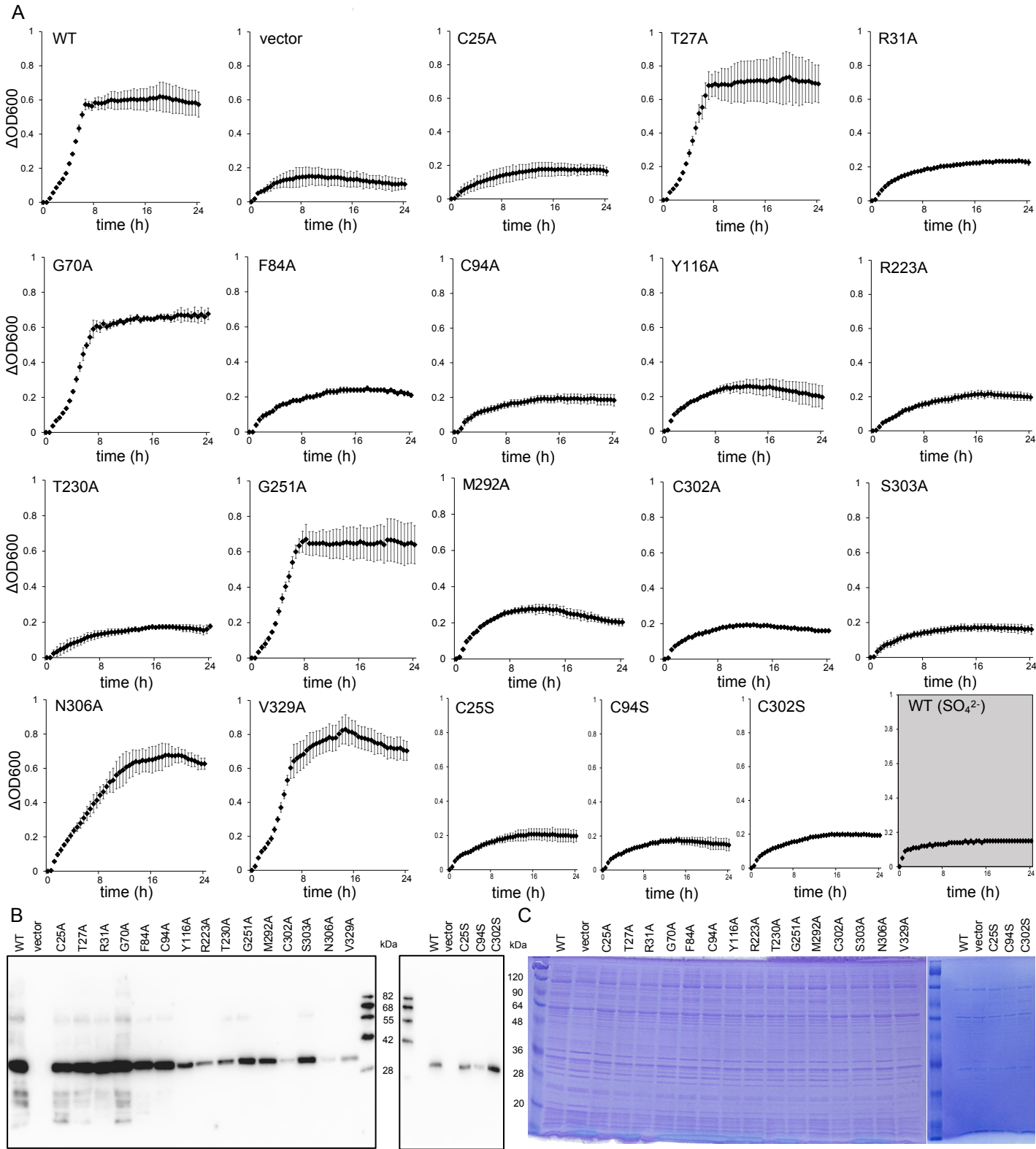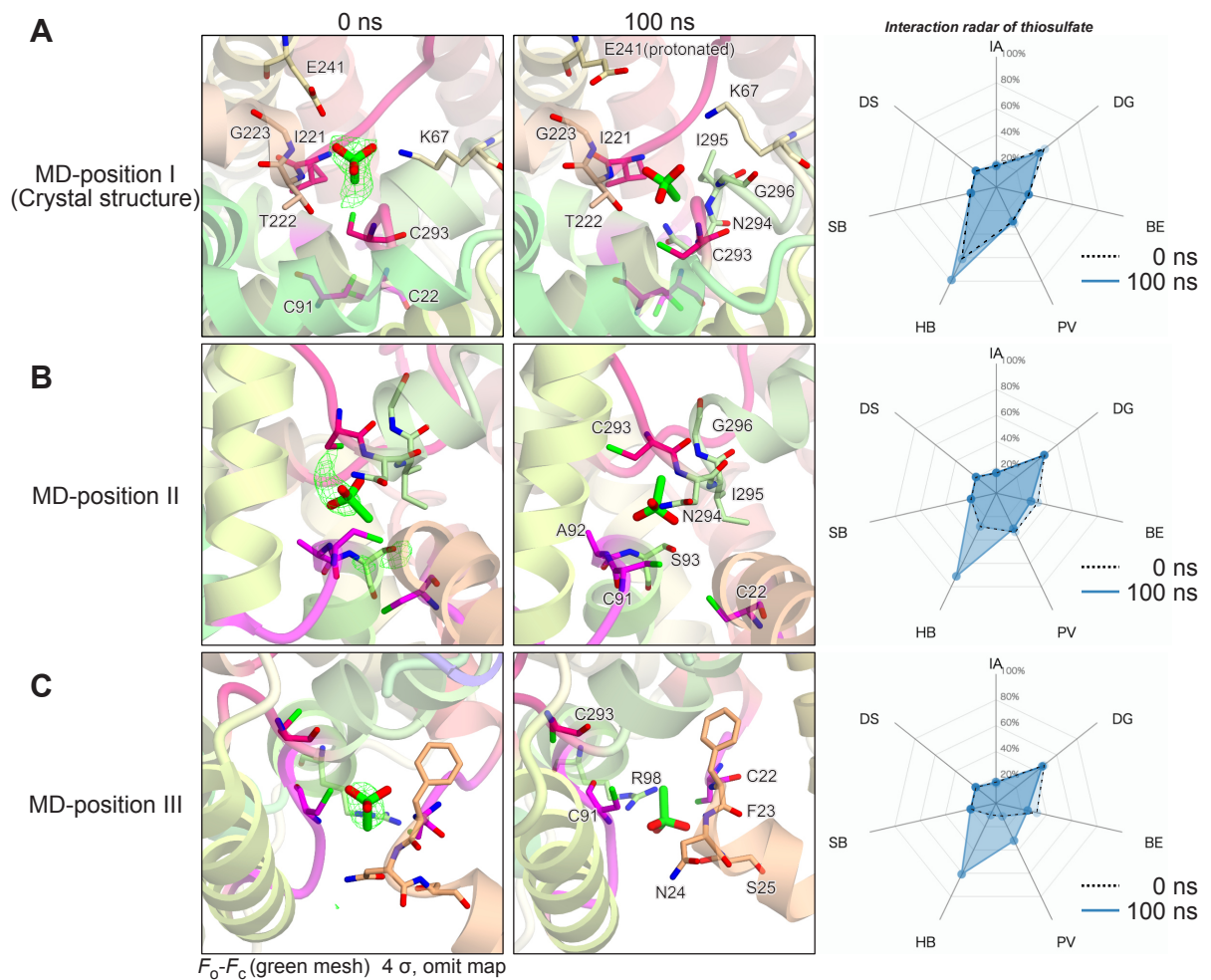
Fig. S4. Original data for Fig. 5A. (**A**) Growth (ΔOD600) of Δ*cysPUWA* Δ*yeeE* (DE3) cells expressing *E. coli* YeeE mutants in single sulfur source minimal media containing thiosulfate or sulfate (lower right) was monitored at 30-min intervals. Error bars indicate the standard deviation (n = 3). (**B**) Accumulation of *E. coli* YeeE in each transformant. (**C**) Coomassie Brilliant Blue staining of *E. coli* samples for B.

Fig. S5. MD simulations of YeeE with thiosulfate. (A–C) Close-up views of thiosulfate at positions I–III at 0 and 100 ns of MD-positions I–III (left and center, respectively). The initial model for MD-position I was the crystal structure of YeeE. The initial models of MD-positions II and III incorporated a thiosulfate at position II and III, respectively, instead of at position I, based on the extra electron density (*Fo-Fc* map, green). The thiosulfate and surrounding amino acid residues are shown by stick representation. The interaction radar (multi-parametric assessment of macromolecular affinity) of thiosulfate was output by the *jsPISA* program (right). (D) Structural comparisons of the crystal structure and the 100 ns models of MD-positions II and III. The RMSD values for the Cα atoms were calculated. The central region contains LA–LD, H2, H5, H9, and H12. The surrounding transmembrane helices are H1, H3, H4, H6–H8, H10, H11, and H13. In the schematic illustration, regions not considered for the calculation are shown in gray.