An *in vitro* DNA Phosphorothioate Modification Reaction

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Running title: DNA Phosphorothioate Modification Reaction

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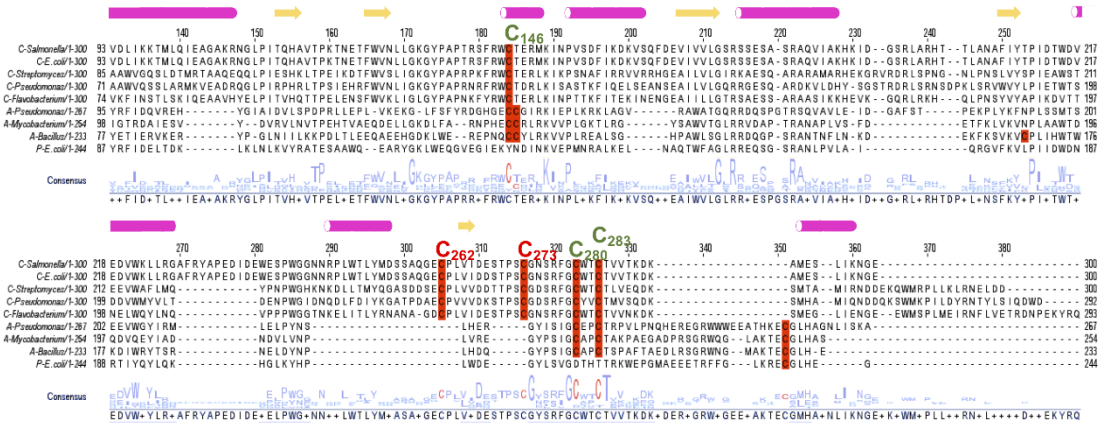

Figure S1 *In silico* analysis of DndC and its homologous proteins

Amino acid sequences of DndC from *Salmonella enterica* serovar Cerro 87, *Escherichia coli* B7A, *Streptomyces lividans*, *Pseudomonas fluorescens*, *Flavobacterium indicum*, and APS reductases from *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Bacillus subtilis*, and PAPS reductase from *E. coli*. were aligned using Jalview2.10.5 (Waterhouse *et al.*, 2009). Residues from 93 to 300 are shown. Cysteine residues are highlighted with red color. Cysteine residue positions in the sequences of DndC from *S. enterica* serovar Cerro 87 are labeled on the top of the residues. The residue C_{146}, C_{280} and C_{283} are involved in the coordination of Fe-S cluster (green color), while the residue C_{262} and C_{273} are not (red color). The secondary structure of DndC was predicted using PSIPRED Protein Analysis Workbench (McGuffin *et al.*, 2000). The predicted α-helixes are shown as fuchsia roundrect, and the predicted β-strands are shown as saffron arrow. Numbers on the right show the actual length of the corresponding amino acid sequences.

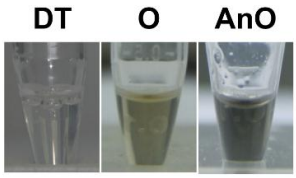

Figure S2 The color of DndCDE

DndCDE was purified in the presence of oxygen as described in the Experimental Procedures section (indicated by 'O'). The protein was then treated using 1 mM α,α'-dipyridyl ('DT'). The Fe-S cluster can be re-constituted under anoxic conditions using the α,α'-dipyridyl treated DndCDE ('AnO').

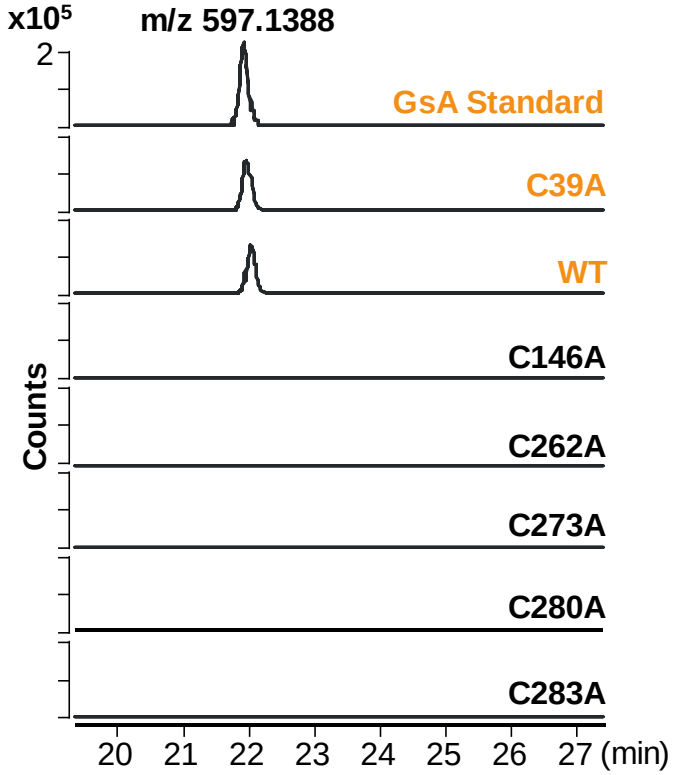

Figure S3 *In vivo* DNA PT modification in *dnd*C mutants detected using HPLC/MS.

The synthesized dGsA standard has a calculated m/z 597.1388.

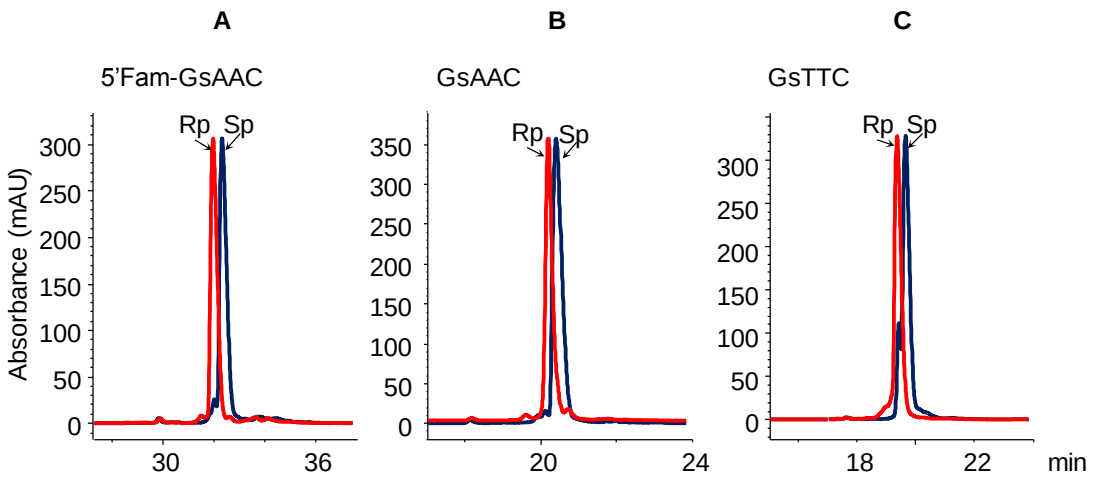

Figure S4 HPLC separation and purification of PT ssDNA of *Rp* and *Sp* configuration.

Synthetic, commercially obtained ssDNA 24 oligonucleotides (DNA1/2 in Table 1) were separated, purified and detected by HPLC. Traces A, B and C show the *Rp* (red) and *Sp* (deep blue) configuration of PT oligonucleotides respectively. Table S1 lists the HPLC conditions for their separation.

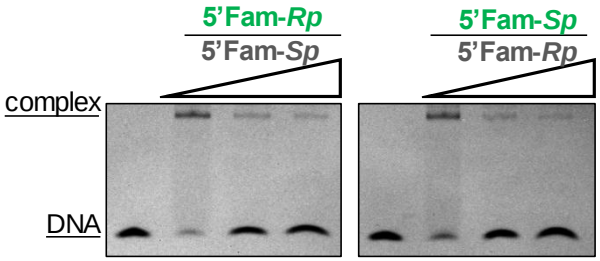

Figure S5 EMSA analysis of DndCDE binding to ds DNA with *Rp* or *Sp* PT modification.

Substrate DNA labeled with fluorescence is indicated by green color. Cold DNA is indicated with gray color. Ten or twenty folds more non labeled cold DNA was used for the experiments.

Table S1. HPLC gradients used to separate *Rp* and *Sp* conformers of phosphorothioated DNA oligonucleotides.

Oligonucleotide	Gradient
24GsAAC (DNA1)	0-5 min, 60 % B*
Fam-24GsAAC (Fam-DNA1)	0-8 min, 70 %-75.2 % B, 8.1-9 min, 75.2 %-70 % B
24GsTTC (DNA2)	0-8 min, 57 %-61.2 % B, 8.1-9 min, 61.2 %-57 % B

* B: solvent B, 10 mM Tris·HCl pH 8.0 and 1 M NaCl. Solvent A was 10 mM Tris·HCl pH 8.0.

References:

McGuffin, L. J., Bryson, K., & Jones, D. T. (2000). The PSIPRED protein structure prediction server. *Bioinformatics,* 16(4), 404-405. doi:10.1093/bioinformatics/16.4.404

Waterhouse, A. M., Procter, J. B., Martin, D. M., Clamp, M., & Barton, G. J. (2009). Jalview Version 2--a multiple sequence alignment editor and analysis workbench. *Bioinformatics,* 25(9), 1189-1191. doi:10.1093/bioinformatics/btp033