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Running title: DNA Phosphorothioate Modification Reaction

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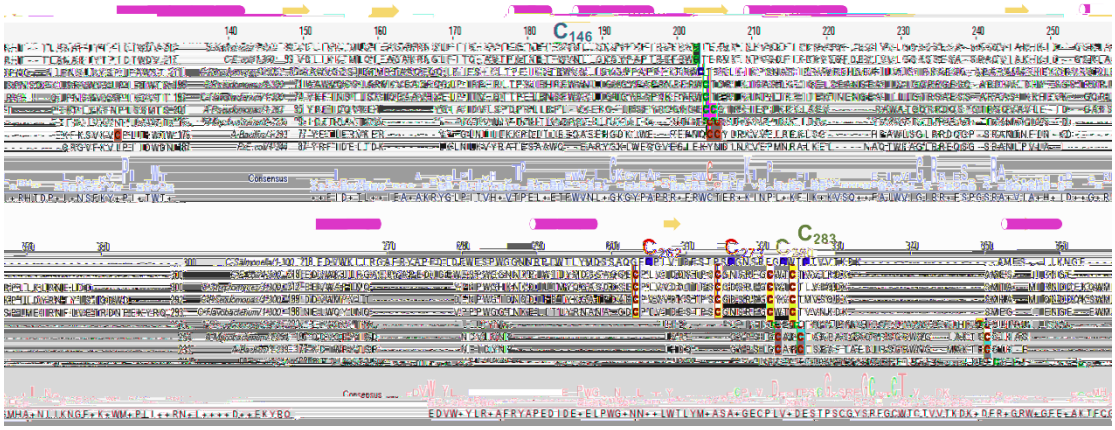

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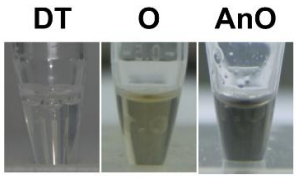
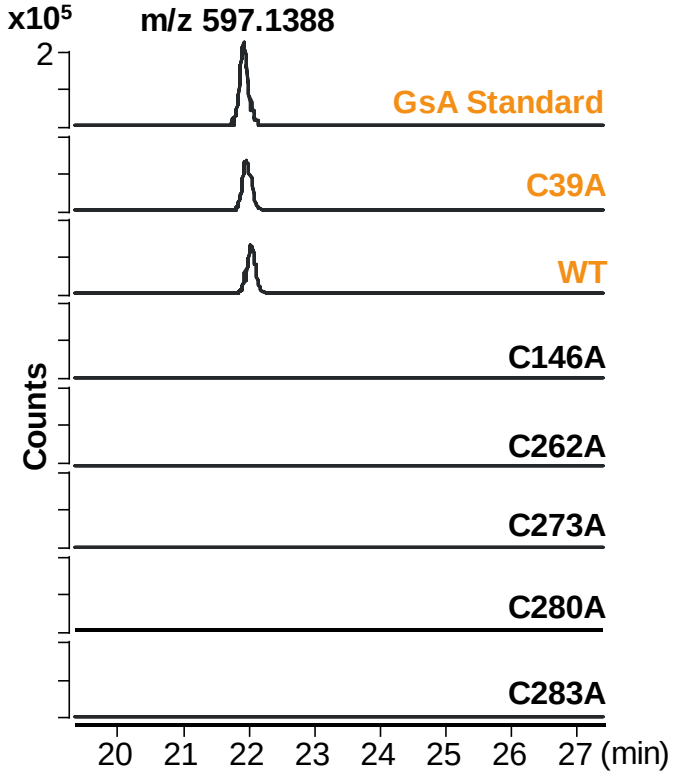
Amino acid sequences of DndC from serovar Cerro 87, B7A, , , , and APS reductases from , , and PAPS reductase from . were aligned using Jalview2.10.5 (Waterhouse , 2009). Residues from 93 to 300 are shown. Cysteine residues are highlighted with red color. Cysteine residue positions in the sequences of DndC from 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 , 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.



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’).



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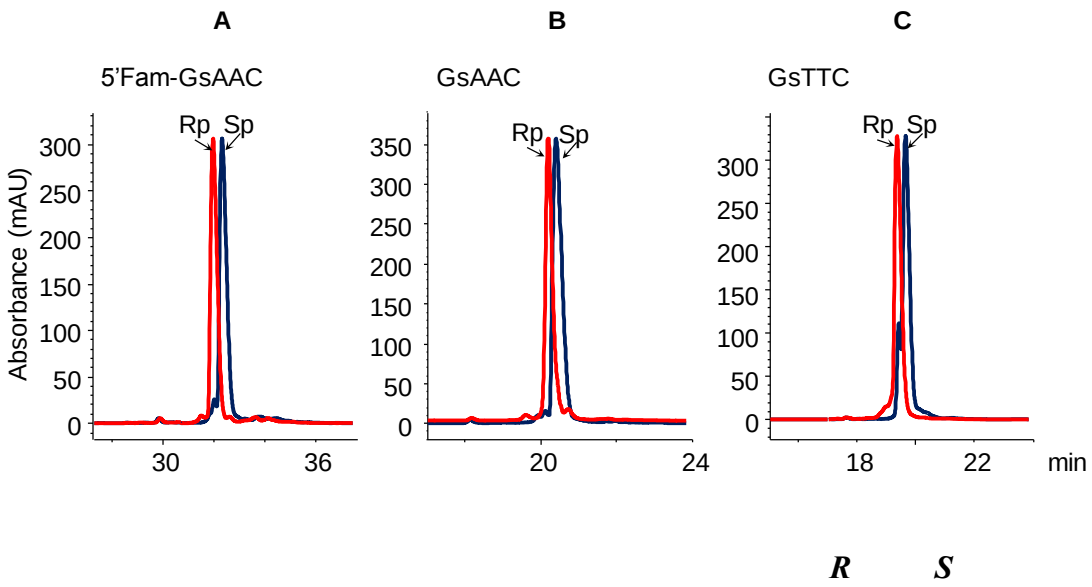
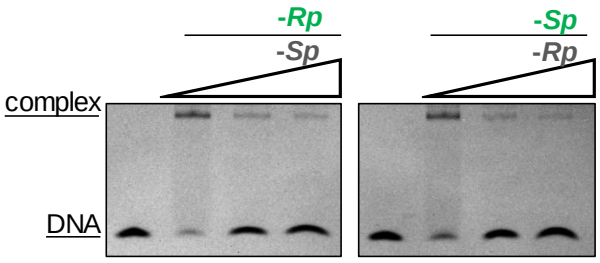
The synthesized dGsA standard has a calculated m/z 597.1388.



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 (red) and (deep blue) configuration of PT oligonucleotides respectively. Table S1 lists the HPLC conditions for their separation.



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.

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:

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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. 25(9), 1189-1191. doi:10.1093/bioinformatics/btp033