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# Structural and Functional Characterization of SoxW-a Thioredoxin Involved in the Transport of Reductants During Sulfur Oxidation by the Global Sulfur Oxidation Reaction Cycle*

[1]Angshuman Bagchi and [2]Tapash Chandra Ghosh
[1]Department of Biochemistry and Biophysics, University of Kalyani,
Kalyani-741235, Nadia, West Bengal, India
[2]Bioinformatics Center, Bose Institute, AJC Bose Centenary Building,
P1/12 CIT Scheme VIIM, Kolkata 700 054, India

**Abstract:** Microbial oxidation-reduction reactions involving inorganic sulfur compounds, mainly the sulfur anions, are one of the vital reactions responsible for the environmental sulfur balance. These reactions are carried out by phylogenetically diverse prokaryotes. These sulfur-oxidizing microbes oxidize inorganic sulfur compounds like sulfide, thiosulfate etc. to produce reductants, which are, involved in the fixation of carbon dioxide or in respiratory electron transport chains. The sulfur oxidizing gene cluster (*sox*) of α-Proteobacteria comprises of at least 15 genes, forming two transcriptional units, viz., *soxSR* and *soxVWXYZABCDEFGH*. SoxW is a periplasmic thioredoxin and an essential component of *sox* operon. It is required for optimal expression of the *sox* gene cluster. All thioredoxins are involved in interaction with DNA polymerase. With the help of docking and molecular dynamics studies we have identified the amino acid residues of the protein involved in the interaction with DNA polymerase to structurally classify SoxW as a thioredoxin. The probable biochemical mechanism of the involvement of the protein in sulfur oxidation has also been investigated. Present study provides a rational basis to interpret the structural classification of SoxW as a thioredoxin and thereby to predict the possible molecular mechanism of the regulation of sulfur anion oxidation reactions by these ecologically important organisms.

**Key words:** Sulfur oxidation, homology modeling, molecular dynamics, protein-protein interaction

## Introduction

Microbial redox reactions of sulfur are mainly responsible for cycling of this element in the environment. Sulfur has a unique range of oxidation states that varies from + 6 to -2 and as a result of which several important biological processes involving transformations of sulfur from one form to other have been evolved. Sulfur based chemo- or photolithotrophy is one of such processes in which electron transfer from reduced sulfur compounds is used by phylogenetically diverse prokaryotes (Friedrich, 1998; Friedrich *et al*., 2005). These sulfur utilizing bacteria oxidize inorganic sulfur compounds like sulfide, thiosulfate etc. to produce reductants that are used for carbon dioxide fixation or in respiratory electron transfer chains (Le Faou *et al*., 1990). The transformation of the sulfur compounds from one form to the other is a major component of the biogeochemical sulfur cycle. Nevertheless, the biochemical mechanism of the bacterial sulfur oxidation process or its regulation is, in general, still poorly understood.

**Corresponding Author:** Angshuman Bagchi, Department of Biochemistry and Biophysics, University of Kalyani, Kalyani-741235, Nadia, West Bengal, India Tel: +91-033-2355-6626 Fax: +91-33-2355-3886

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The sulfur oxidizing gene cluster (*sox*) of α-Proteobacteria comprises of at least 15 genes, which form two transcriptional units, viz., *soxSR* and *soxVWXYZABCDEFGH*. Recent studies with both chemo- and photolithotrophic α-Proteobacteria such as *Paracoccus pantotrophus* (Para) and *Rhodovulum sulfidophilum* (Rsulf) revealed that multiple-gene cluster, *shxVW* (*soxVW*) and *soxXYZABCDEFGH*, is associated with the metabolism sulfur anions (Appia-Ayme *et al*., 2001; Rother *et al*., 2001). SoxXA, SoxYZ, SoxB and SoxCD are required for sulfur-dependent cytochrome *c* reduction. The eight-electron oxidation of a molecule of thiosulfate is governed by cytochrome *c* complex multienzyme system (TOMES) encoded by *soxXYZABCD*. The enzyme system including, ShxV and ShxW, which were proposed to be involved in the biogenesis of *cytochrome c*, were shown to be inducible by thiosulfate (Appia-Ayme *et al*., 2001; Rother *et al*., 2001). SoxV was shown to function in the reduction of the periplasmic protein SoxW, which shows a CysXaaXaaCys motif characteristic for thioredoxins (Appia-Ayme *et al*., 2002). Though a considerable progress in the genetics of sulfur lithotrophy is noted, molecular mechanism of regulation of *sox* gene expression has not yet been addressed. In the present study we seek to understand the potential of SoxW protein as the mediator of electron transport in the *sox* operon. We describe the three-dimensional structure of SoxW. Since SoxW is a thioredoxin and generally thioredoxins interact strongly with DNA polymerase (Bhattacharya *et al*., 2002), we have used molecular docking and molecular dynamics in order to structurally classify SoxW as a thioredoxin. The redox active binding site of SoxW has been predicted, analyzed and compared to that of previously reported experimental results. These studies provide detailed structural information on the binding of SoxW as well as its mode of action. As this is the first report regarding the structural basis of the involvements of SoxW in the process of biochemical oxidation of sulfur anions, our studies may contribute towards the understanding of the molecular mechanism of sulfur anion oxidation by these ecologically important microbial species.

## Materials and Methods

*Docking and Molecular Dynamics Calculations*

The three dimensional coordinates of SoxW were obtained from the protein data bank (Berman *et al*., 2000) (pdb code: 2H0O) (Bagchi and Ghosh, 2006 ). It is well known that thioredoxin proteins interact with DNA polymerase (Bhattacharya *et al*., 2002). To study the interaction between SoxW and DNA polymerase, the coordinates of the DNA polymerase bound to thioredoxin (pdb code: 1TKD) protein were extracted from PDB. The coordinates of the thioredoxin motif of SoxW were superimposed on the corresponding motif of the thioredoxin protein bound to DNA polymerase in 1TKD. The rmsd of the superimposition was 0.4 Å. The model of SoxW was subsequently merged with the crystal structure of 1TKD and the thioredoxin protein bound to the DNA polymerase was then removed to form a SoxW-DNA-polymerase complex. The model of SoxW was also docked to DNA-polymerase using the software GRAMM (Vakser, 1995) and ZDOCK (Chen and Weng, 2003), using the ClusPro server (Comeau *et al*., 2004) and also with Patchdock server (Schneidman-Duhovny *et al*., 2003) in order to get a comprehensive result. The docked structures, that yielded the best score were selected and analyzed visually using the software Insight II. Molecular dynamics (MD) simulations were performed on the docked structures to predict the favourable binding interactions. The docked structures were solvated with an average of 2000 simple point charges (Berendsen *et al*., 1981) water molecules. The system was minimized initially keeping the water and the backbones of the proteins fixed. In the next step of minimization, the protein complex was kept fixed and the water molecules were allowed to move. The first few rounds of minimizations were performed by steepest descent method and then conjugate gradient method was employed. The minimized system was equilibrated for a period of 10 ps with positional restraints. Then a 120 ps MD run was performed without restraints. Weak coupling of the protein to a solvent bath of constant

temperature (300 K) and constant pressure (1 bar) was maintained with a coupling of 1.0 ps. All energy minimizations and MD simulations were performed using Insight II molecular simulation package. All the structures were finally analyzed by PROCHECK (Laskowski *et al*., 1993).

*Calculations for Protein-protein Interactions*

To find out the interactions between the protein complex (i.e., SoxW-1TKD), What If software package (Vriend, 1990) as well as the Biopolymer module of Insight II were used. These programs calculate the interactions between two groups by measuring the distance between them.

## Results and Discussion

*Description of the Structure of SoxW*

SoxW consists of 187 amino acid residues. The protein is made up of a four-stranded β-sheet sandwiched between two helices on one side and third on the other. There are two parallel and two anti-parallel β-sheets. The overall topologic arrangement of the secondary structural elements is like β-α-β-α-β-β-α- (Fig. 1). The first helix of the protein (amino acid residues 42 to 48) is connected to the β-sheet by a short coil. Thioredoxin motif of the protein that comprises of the first β-strand (amino acid residue 53 to 60) is connected to the second helix (amino acid residue 65 to 70). When the 3D coordinates of the modeled SoxW were used to search structural homologs using DALI (Holm and Sander, 1997), the search results showed that the top hits (except the templates 1VRS and 1XYC) belong to known thioredoxin proteins. The redox active site (Cys64-Ile65-Tyr66-Cys67) is

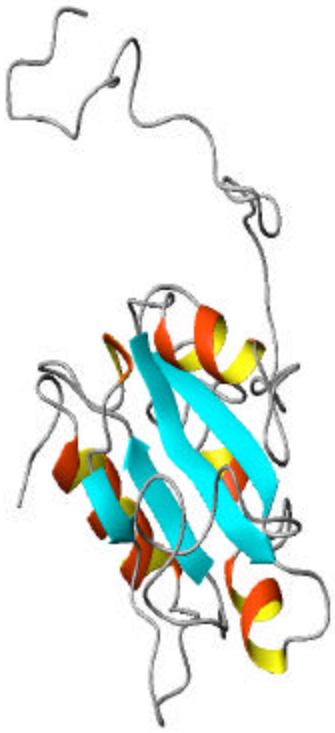

Fig. 1: Ribbon representation of modeled SoxW. α-helices and β-sheets are shown as helices (Red and Yellow) and ribbons (Cyan), respectively. The rest are shown as loops (White). The figure was prepared by MOLSCRIPT (Kraulis, 1991)

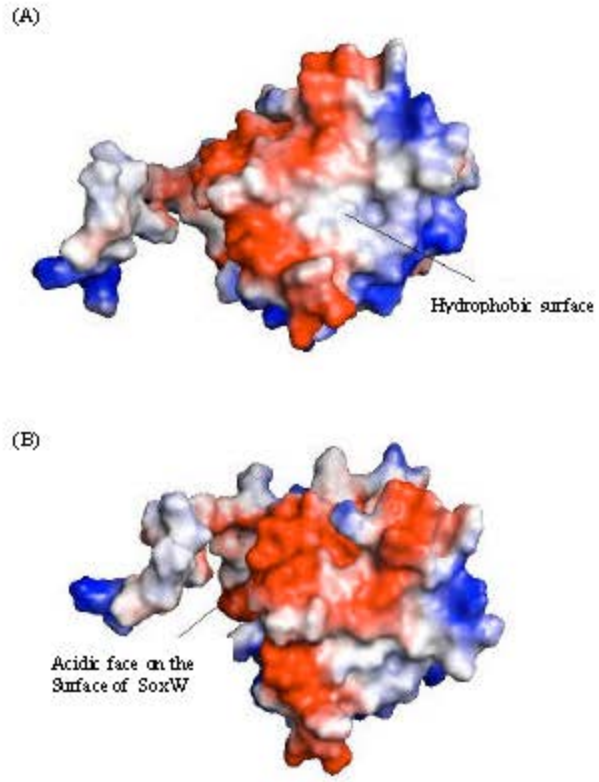


Fig. 2: Electrostatic surface potentials of SoxW showing the exposed hydrophobic surface on one side (A) and an acidic surface on the opposite face (B)

solvent accessible on one side of the molecule but solvent inaccessible on the other. A close inspection of the active site structure of the protein reveals that the side chains of Trp59, Gln61, Gly63, thr68 and Met 70 effectively block any approach to Cys 64. The opposite to this redox active site there is a hydrophobic patch containing Cys 64, Ile 65, Val 74, Phe 75, Pro 76, Pro 78, Val 99 and Val 101 (Fig. 2) as observed in case of other well defined thioredoxins (Bhattacharya *et al*., 2002).

*Structural Comparison with Other Thioredoxins*

In order to carry out the structural comparison with other thioredoxins, the coordinates of SoxW were deposited to DALI (Holm and Sandar, 1997) and CE databases (Shindyalov and Bourne, 2001). The top hits from these searches, assessed by their Z-scores, number of matching residues and RMSD values, belong to well-defined thioredoxin proteins. Figure 3 represents the structure based sequence alignment between SoxW and other well characterized thioredoxin proteins. The Fig. 3 depicts the relative positioning of the secondary structural elements. The alignment reflects the similarity in the packing of the secondary structural elements in the proteins.

Moreover, superimpositions of the thioredoxin motif of SoxW onto the corresponding motifs of other well characterized thioredoxin proteins 1THX, 4TRX, 3TRX and 1ERV produced r.m.s deviations of 0.6, 0.7, 0.7 and 0.2 Å, respectively. This shows that the overall topological arrangements of the secondary structural elements of SoxW are similar to these well-characterized thioredoxin family proteins (Fig. 4). Moreover the backbone conformation of the redox active motif of SoxW was similar

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SoxW        HKPDWLRQTFRDMREDLAEAEAENRRMLVIWEQRGCIY-CTRMHEEVFPDPEIEALIRER
1SRX        SDKIIHLT---DDSFDTDLVKADGAI-LVDFWAEWCGP-CKMIA---PILDEIAD----E
2TRX        --IIH---LTD-DSFDTDVLKAD-GAILVDFWAEWCGP-CKMIA---PILDEIAD----E
2TIR        --IIH---LTD-DSFDTDVLKAD-GAILVDFWAEWCGP-CEMIA---PILDEIAD----E
1TXX        --IIH---LTD-DSFDTDVLKAD-GAILVDFWAEWCVW-CKMIA---PILDEIAD----E
1THO        --IIH---LTD-DSFDTDVLKADG-AILVDFWAEWCGRPCKMIA---PILDEIAD----E
1T7P        --IIH---LTD-DSFDTDVLKA-DGAILVDFWAEWCGP-CKMIA---PILDEIAD----E
2TRX        --IIH---LTD-DSFDTDVLKAD-GAILVDFWAEWCGP-CKMIA---PILDEIAD----E
1X0B        --IIH---LT-DDSFDTDVLKAD-GAILVDFWAEWCGP-CKMIA---PILDEIAD----E

SoxW        YFVVQM---N----LFGDVEVT-------D-FDGTVLPEKEMAGRWGVMFTPTLMFMPET
1SRX        YQ---G---K----LTVAKLNI-------DQNPGTAP---KYI--ERGIPTLLLFKNG--
2TRX        YQ----G--K----LTVAKLNID------Q-NPGTAP---KYGIR--GIPTLLLF-----
2TIR        YQ-------GK---LTVAKLNI-D-----Q-NPGTAP---KYGIR--GIPTLLLF-----
1TXX        YQ-------G-K--LTVAKLNI--D----Q-NPGTAP---KYGIR--GIPTLLLF-----
1THO        YQ-------G--K-LTVAKLNI---D---Q-NPGTAP---KYGIR--GIPTLLLF-----
1T7P        YQ-------G---KLTVAKLNI----D--Q-NPGTAP---KYGIR--GIPTLLLF-----
2TRX        YQ-----G-K----LTVAKLNI-----D-Q-NPGTAP---KYGIR--GIPTLLLF-----
1X0B        YQ-----GK----LTVAKLNI------DQ-NPGTAP---KYGIR--GIPTLLLF-----

SoxW        PPEGGTAAEAAVASMPGAFGK------G-TTRALLQWVLD
1SRX        ----EVAATKV-----GALSK------G-QLKEFLDANLA
2TRX        -KNGEVAATKV-----GALS-K-----G-QLKEFLDANL-
2TIR        -KNGEVAATKV-----GALS--K----G-QLKEFLDANL-
1TXX        -KNGEVAATKV-----GALS---K---G-QLKEFLDANL-
1THO        -KNGEVAATKV-----GALS----K--G-QLKEFLDANL-
1T7P        -KNGEVAATKV-----GALS-----K-G-QLKEFLDANL-
2TRX        -KNGEVAATKV-----GALS------KG-QLKEFLDANL-
1X0B        -KNGEVAATKV-----GALS-------KGQLKEFLDANL-
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Fig. 3: Structure-based sequence alignment of SoxW with other well characterized thioredoxin family proteins

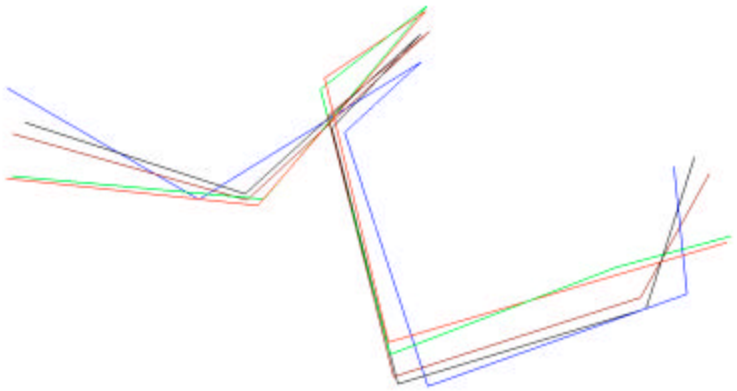

Fig. 4: Superimposition of the α-carbon backbones of the thioredoxin motif of SoxW (Black) on 1THX (Blue), 4TRX (Green), 3TRX (Red) and 1ERV (Brown)

to the well-characterized thioredoxins (Table 1). Electrostatic potential mapped onto the surface of the proteins in the unit of kT/e shows the similarity between the charge distributions of the proteins (Fig. 5). All the proteins have a similar distribution of positive and negative charges on their surfaces.

*Interaction of SoxW with T7 DNA Polymerase*

All the thioredoxins interact with DNA polymerase in a similar way (Bhattacharya *et al*., 2002). To assess if SoxW interacts favourably with DNA polymerase as observed in other thioredoxins and to structurally classify it as a thioredoxin, we docked SoxW on to DNA polymerase. The protein-protein interface is found to contain mainly the polar amino acid residues. The interior of the complex is made

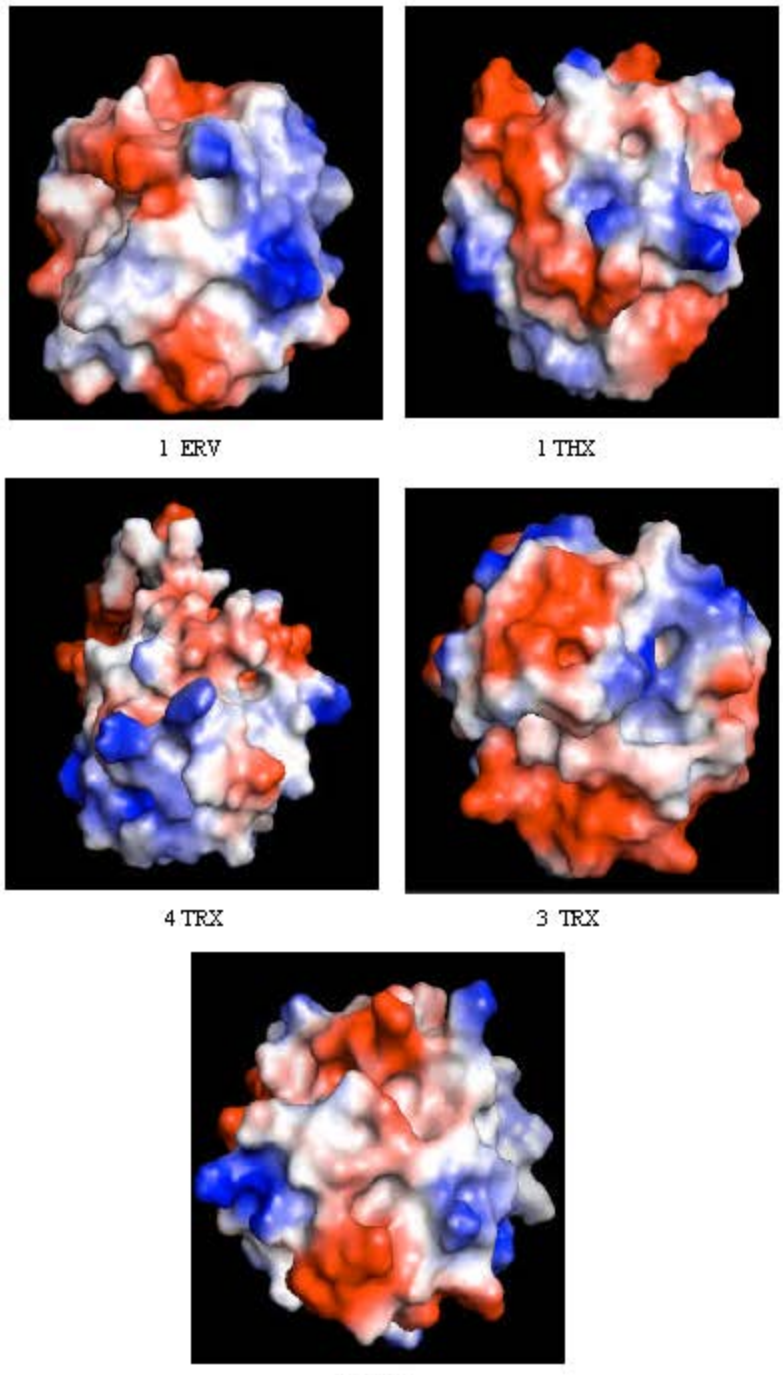


Fig. 5: Electrostatic surfaces of 1THX, 4TRX, 3TRX, 1ERV and SoxW

Table 1: Comparison of the φ and ψ angles of the active site residues of SoxW with other known thioredoxins

| Protein | Amino acid | φ | ψ |
|---|---|---|---|
| 1ERV | Cys32 | -92.8 | 120.8 |
| | Gly33 | -49.0 | -62.0 |
| | Pro34 | -54.0 | -27.0 |
| | Cys35 | -67.3 | -45.1 |
| 1THX | Cys32 | -99.7 | 112.2 |
| | Gly33 | -58.4 | -54.0 |
| | Pro34 | -59.7 | -31.1 |
| | Cys35 | -62.2 | -48.8 |
| 2TRX | Cys32 | -90.2 | 118.7 |
| | Gly33 | -60.7 | -53.6 |
| | Pro34 | -59.4 | -30.1 |
| | Cys35 | -63.5 | -44.6 |
| 3TRX | Cys32 | -89.0 | 126.2 |
| | Gly33 | -56.8 | -61.5 |
| | Pro34 | -57.4 | -30.9 |
| | Cys35 | -63.7 | -44.1 |
| SoxW | Cys64 | -96.0 | 110.2 |
| | Ile65 | -66.2 | -60.9 |
| | Tyr66 | -61.4 | -38.9 |
| | Cys67 | -70.7 | -43.1 |

Table 2: Comparative results of Binding Interactions of SoxW and *E. coli* thioredoxin with DNA polymerase

| Complex | ΔH (Kcal/mol) | TΔS (Kcal/mol) | ΔG (Kcal/mol) |
|---|---|---|---|
| DNApol-EcTrx (X-ray)[a] | 3.74 | 27.53 | -23.79 |
| DNApol-EcTrx[b] | 3.99 | 28.25 | -24.26 |
| DNApol-SoxW[c] | 3.67 | 27.83 | -24.16 |

T = 300 K, [a]X-ray crystal structure of DNA polymerase bound to *E. coli* Trx (pdb code: 1T7P), [b]Energy-minimized crystal structure of DNA polymerase bound to *E. coli* Trx, [c] Energy-minimized structure of DNA polymerase bound to SoxW

up of hydrophobic amino acids. There are extensive Hydrogen bonding (H-bonding) interactions between the two protein molecules. The main chains of Val44, Ser146 and Pro148 form H-bond with the main chains of Glu319, Pro292 and Ala324 of DNA polymerase respectively as observed in other well-characterized thioredoxins (Bhattacharya *et al*., 2002). The backbone amide and carbonyl groups of the active site residues of SoxW are also involved in charge-charge interactions between the proteins. Moreover, the binding interactions of the complex were quantitatively evaluated by calculating the accessible surface area and thermodynamic parameters using the Structural Thermodynamics Calculator (STC) program (Lavigne *et al*., 2000). The calculated free energy change on binding between SoxW with DNA polymerase is found to be comparable to the interactions between other well characterized thioredoxins with DNA polymerase (Table 2). These results may justify that SoxW interacts similarly with DNA polymerase as other well-known thioredoxins. Considering all these points we could safely conclude that SoxW belongs to the family of thioredoxins.

## Conclusions

We have made an attempt to structurally classify SoxW as a thioredoxin. We describe the three dimensional structure of the SoxW. Since all thioredoxins interact with T7 DNA polymerase, we docked SoxW to DNA polymerase and identified the residues involved in the interaction. With the help of sequence and thermodynamic analysis of the SoxW and SoxW-DNA polymerase complex we could establish SoxW as thioredoxin. The probable role of SoxW in biochemical sulfur anion oxidation was also predicted. Finally we note that our studies will help in designing experiments for

future genetic and biochemical work in order to elucidate the role of different residues of SoxW in efficient regulation of *sox* gene expression in these ecologically important sulfur-oxidizing microorganisms.

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