



**HAL**  
open science

## **SORGOdb: Superoxide Reductase Gene Ontology curated DataBase.**

Céline Lucchetti-Miganeh, David Goudenège, David Thybert, Gilles Salbert,  
Frédérique Barloy-Hubler

► **To cite this version:**

Céline Lucchetti-Miganeh, David Goudenège, David Thybert, Gilles Salbert, Frédérique Barloy-Hubler. SORGOdb: Superoxide Reductase Gene Ontology curated DataBase.. BMC Microbiology, 2011, 11, pp.105. 10.1186/1471-2180-11-105 . hal-00605708

**HAL Id: hal-00605708**

**<https://hal.science/hal-00605708>**

Submitted on 4 Jul 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

DATABASE

Open Access

# SORGOdb: Superoxide Reductase Gene Ontology curated DataBase

Céline Lucchetti-Miganeh<sup>1\*</sup>, David Goudenège<sup>1</sup>, David Thybert<sup>1,2</sup>, Gilles Salbert<sup>1</sup> and Frédérique Barloy-Hubler<sup>1</sup>

## Abstract

**Background:** Superoxide reductases (SOR) catalyse the reduction of superoxide anions to hydrogen peroxide and are involved in the oxidative stress defences of anaerobic and facultative anaerobic organisms. Genes encoding SOR were discovered recently and suffer from annotation problems. These genes, named *sor*, are short and the transfer of annotations from previously characterized neelaredoxin, desulfoferrodoxin, superoxide reductase and rubredoxin oxidase has been heterogeneous. Consequently, many *sor* remain anonymous or mis-annotated.

**Description:** SORGOdb is an exhaustive database of SOR that proposes a new classification based on domain architecture. SORGOdb supplies a simple user-friendly web-based database for retrieving and exploring relevant information about the proposed SOR families. The database can be queried using an organism name, a locus tag or phylogenetic criteria, and also offers sequence similarity searches using BlastP. Genes encoding SOR have been re-annotated in all available genome sequences (prokaryotic and eukaryotic (complete and in draft) genomes, updated in May 2010).

**Conclusions:** SORGOdb contains 325 non-redundant and curated SOR, from 274 organisms. It proposes a new classification of SOR into seven different classes and allows biologists to explore and analyze *sor* in order to establish correlations between the class of SOR and organism phenotypes. SORGOdb is freely available at <http://sorgo.genouest.org/index.php>.

## Background

Two and a half billion years ago, the intense photosynthetic activity of *cyanobacteria* caused the largest environmental change in Earth's history: the oxygenation of the atmosphere and the oceans, which were hitherto largely anoxic [1,2]. This profound transformation of the biosphere exerted an evolutionary selection pressure on organisms and led to the development of new pathways, including the highly exergonic respiratory chain based on O<sub>2</sub> as the terminal electron acceptor. Currently, most living organisms, except anaerobic microbes, require oxygen. O<sub>2</sub> is used as a substrate by many enzymes involved metabolizing amines, purines and amino acids. Oxygen is a relatively inert molecule due to its spin triplet ground state. However, it can be activated by photons or by one electron oxidation or reduction processes to generate reactive oxygen species

(called reactive oxygen species or ROS), particularly hydroxyl radicals ( $\bullet$ OH), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and superoxide anion radicals (O<sub>2</sub><sup>-</sup>).

The superoxide anion is generated fortuitously by flavoenzymes such as NADH dehydrogenase II, succinate dehydrogenase, fumarate reductase, and sulphite reductase [3,4]. The superoxide anion is one of the deleterious reactive oxygen species: it can damage DNA, proteins and lipids indirectly by releasing iron from damaged dehydratase clusters [4,5]. In anaerobes, most of the essential "central metabolic" redox enzymes (for example aconitase, fumarase, dihydroxyacid dehydratase, and pyruvate:ferredoxin oxidoreductase) contain iron sulphur [Fe-S] clusters that are rapidly inactivated when exposed to oxygen [5-8].

To survive and protect themselves from the toxicity of superoxide anion, many species, and especially anaerobes, have developed defence mechanisms [5].

Superoxide dismutase (SOD) was first isolated by Mann and Keilis (1938) and its catalytic function, which consists to dismutate O<sub>2</sub><sup>-</sup> into molecular oxygen and

\* Correspondence: [celine.lucchetti@univ-rennes1.fr](mailto:celine.lucchetti@univ-rennes1.fr)

<sup>1</sup>CNRS UMR 6026, ICM, Equipe Sp@rte, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes, France

Full list of author information is available at the end of the article

hydrogen peroxide, was discovered in 1969 by McCord and Fridovich [9]. Mammals have two forms of SOD isozymes: the manganese SOD (Mn-SOD), present in the mitochondria, and the copper/zinc SOD (Cu/Zn-SO), present in the cytoplasm [10,11]. In plants, SOD have been classified into three distinct types on the basis of their metal cofactor: Cu/Zn-SOD (in the cytosol and chloroplasts), Mn-SOD (in mitochondria), and Fe-SOD (often in chloroplasts) [12-14]. There are three known SOD in *E. coli*: MnSOD, FeSOD and CuZnSOD. The two first are located in the cytoplasm and the last in the periplasmic space [15]. A distinct additional fourth class of SOD containing nickel (NiSOD) was recently discovered in *Streptomyces* [16,17] and cyanobacteria [18]. SOD-driven dismutation was the only biological mechanism identified for scavenging superoxide anion radicals until the early 1990's. McCord *et al.* [19] established a correlation between oxygen tolerance and SOD production and suggested that SOD was the single most important enzyme for enabling organisms to survive in the presence of molecular oxygen. They proposed that the hypersensitivity of obligate anaerobes to oxygen was a consequence of SOD deficiency. However, most anaerobic organisms, which indeed lack SOD, show various degrees of tolerance to oxygen when they are occasionally exposed to this molecule in their environments.

Two novel iron-sulphur-containing proteins that detoxify superoxide molecules were then discovered in sulphate-reducing and hyperthermophilic anaerobes: desulfoferrodoxin (Dfx) in *Desulfovibrio desulfuricans*, *Desulfovibrio vulgaris Hildenborough* [20] and *Desulfoarculus baarsii* [21], neelaredoxin (Nlr) in *Desulfovibrio gigas* [22] and superoxide reductase (SOR) in *Pyrococcus furiosus* [23]. This revealed the existence of alternative mechanisms for ROS detoxification in anaerobes. The function of these proteins was first studied in 1996 by Dfx complementation of superoxide detoxication activity in *E. coli* SOD mutants [24]. Later, Nlr from *Treponema pallidum* [25] and *D. gigas* [26] were also shown to complement such SOD mutants. Liochev and Fridovich [27] suggested that Dfx catalyzes the reduction of superoxide rather than its dismutation, and that it uses cellular reductants such as NAD(P)H. Subsequently, the Dfx enzyme was confirmed as an oxidoreductase [23-25,27]. Finally, the superoxide reductase activity of those proteins were established by two groups [21,23].

Dfx and Nlr proteins have different numbers of iron sites: both contain a similar C-terminal single iron-containing site (centre II) but also has Dfx a second N-terminal site (centre I) [22,28]. Centre II is the active site of SOR and consists of a pentacoordinated Fe<sup>2+</sup> centre with four equatorial histidines and one axial cysteine in a square pyramidal geometry (Fe(His)<sub>4</sub>(Cys) [29-31]). The binding site for the substrate O<sub>2</sub><sup>-</sup> is the free sixth

axial site of the reduced enzyme centre [30]. The additional N-terminal domain of the 2Fe-SOR contains a rubredoxin-like centre, with Fe<sup>3+</sup> ligated by four cysteines in a distorted tetrahedral geometry (centre I, Fe(Cys)<sub>4</sub>, [32]). A first classification of these enzymes was proposed according to the number of metal centres: neelaredoxin or 1Fe-SOR and desulfoferrodoxin or 2Fe-SOR [33,34]. An additional class was proposed after the isolation of a *Treponema pallidum* SOR that contains an extended non-iron N-terminal domain of unknown function [25,35]. In all these three classes, only the reduced form of the iron-containing active centre II is able to react with the superoxide anion O<sub>2</sub><sup>-</sup>.

SOD are found in nearly every living organism except in some strictly anaerobic species [36,37]. Tally *et al* suggested that the diversity in the oxygen tolerance of anaerobes is generally related to their level of SOD [38]. SOR were first thought to be restricted to anaerobic prokaryotes but were subsequently discovered in some micro-aerophilic and micro-aerotolerant Bacteria and Archaea [39,40]. More recently, a SOR encoding gene was also discovered in an eukaryote, *Giardia intestinalis*, a microaerophilic protozoan (cited by [41]). Although SOD and SOR both detoxify superoxide, there is a fundamental difference in their properties: SOD generate one-half mole of oxygen and one-half mole of hydrogen peroxide per superoxide molecule whereas SOR produce only one mole of hydrogen peroxide. The physiological conditions, that determine SOR or SOD preference in organisms, have not be completely determined, although the presence of SOR rather than SOD may be associated with the amount of redox proteins produced by organisms [25].

Most genomes, even those of anaerobic species, contain both SOD and SOR although some species have only one of the two enzymes. The increasing number of sequenced genomes makes allows comparative genomic analyses, to elucidate the evolutionary or functional processes of SOR. Unfortunately, there are several problems with the annotation of superoxide reductase genes, partly a consequence of heterogeneous transfer of annotations from previously characterized neelaredoxin, desulfoferrodoxin, superoxide reductase or rubredoxin oxidase. Moreover, due to the absence of updating or correction of databases, many *sor* genes remained anonymous because of the transfer of annotations from SOR genes initially annotated as "hypothetical", "function unknown" or "putative activity". Also, SOR are small proteins, ca. 200 amino acids on average, and mis-annotations are frequent for proteins of this length [42].

For all these reasons, we developed SORGOdb, the first resource specifically dedicated to superoxide reductase genes in entirely sequenced and in-draft genomes. SOR sequences were curated manually, analysed and

stored using a new ontology in a publically available resource (<http://sorgo.genouest.org/>). SOR genes were detected in the three kingdoms of life, and only on chromosomal replicons. Although no N-terminal signal sequences were previously described for bacteria SOR [43], we predicted seven SOR to be potentially TAT-secreted (Twin-arginine translocation) in some bacteria, including for example in *Desulfovibrio salexigens* DSM 2638, *Desulfuromonas acetoxidans* DSM 684 and *Geobacter uraniireducens* Rf4. Our analysis confirms the observations by Pinto et al in 2010 that (1) the repartition of SOR classes does not correlate with organism phylogeny and that (2) sor genes occur in very diverse genetic environments. Indeed, although some sor are clustered with genes encoding electron donors (such as rubredoxin in *D. vulgaris*) or inter-related oxidative responsive genes, most are close to functionally unrelated genes. This is consistent with sor genes being acquired, or lost, through lateral gene transfer [41].

## Construction and content

### Collection of SOR

For collection of SOR, we have extensively searched the Pubmed database and identified all relevant literature concerning any protein with “superoxide reductase” activity; this search resulted in a small dataset (13 SOR published in 12 organisms, see Table 1). We therefore enriched the database using manually curated sequences described as desulfoferrodoxin (160 proteins), superoxide reductase (50 proteins) or neelaredoxin (9 proteins) in EntrezGene and/or GenBank entries. As the “centre II” is the active site for the SOR activity, we also included all proteins with a domain of this type as described in InterPro (IPR002742, IPR004793, IPR004462, IPR012002), Pfam (PF01880, PF06397),

Supfam (SSF49367), TIGRfam (TIGR00332, TIGR00320, TIGR00319), NCBI conserved domains (cd03172, cd03171, cd00524, cl00018, cl00014, cd00974) and PRODOM (PD006618, PD330262, PDA2O7Z7, PDA36750, PD985590, PDA36751, PDA63215, PDA7Y161, PDA7Y162, PD511041, PD171746, PD985589, PDA7Y163). All sequences collected were cleaned up to remove redundancy and unrelated proteins. This non-redundant and curated dataset was used to investigate the 1237 complete and 1345 in-draft genomes available in the NCBI database (May, 2010) through a series of successive BlastP [44] and tBlastN [45] searches. Orthology (KO K05919 and COG2033) and synteny (IMG neighbourhood interface) were also exploited. To be as comprehensive as possible in the data collection, we performed multiple alignments using both ClustalW [46,47] and Muscle [48] algorithms. These alignments showed highly conserved residues in the sequences of active centre I (CX<sub>2</sub>CX<sub>15</sub>CC) and centre II (HX<sub>5</sub>H-CX<sub>2</sub>H). These conversations were translated into “regular expressions” that were used to perform for final screening of databases. All these search processes allowed us to retrieve 106 supplementary proteins including 82 proteins described as “hypothetical protein”.

At the end of this integrative research, we had a collection of 325 non-redundant and curated predicted SOR in 274 organisms, covering all the three kingdoms: Bacteria (270 genes), Archaea (52 genes) and Eukaryota (3 genes).

### New Classification and ontology

Consistent with the collecting procedure, all the 325 proteins present in SORGOdb contain at least the SOR active centre II domain. However, we found that this SOR module is, in some cases, associated with other domains, in a modular way. The discovery of new

**Table 1 SOR proteins with entrie(s) in Pubmed and/or PDB structure**

Organism	Locus Tag	PDB	PMID
<i>Desulfovibrio desulfuricans</i> ssp. <i>desulfuricans</i> ATCC 27774	Ddes_2010	1DFX	[20,56,76-78]
<i>Desulfovibrio Desulfuricans</i> ssp. <i>desulfuricans</i> G20	Dde_3193	2JI3, 2JI2,	[79]
<i>Desulfoarculus baarsii</i>	rbo	2JI1, 1VZI, 1VZG, 1VZH	[25,52,79-87]
<i>Pyrococcus horikoshii</i> Ot3	PH1083	2HVB	[30]
<i>Pyrococcus furiosus</i> DSM 3638	PF1281	1DQI, 1DO6, 1DQK	[29,30,88-91]
<i>Treponema pallidum</i> ssp. <i>pallidum</i> str. <i>Nichols</i>	TP0823	1Y07	[21,35,52,82,86,92-99]
<i>Treponema maritima</i>		2AMU	
<i>Archaeoglobus fulgidus</i> DSM 4304	AF0833, AF0344		[51,55,100-103]
<i>Desulfovibrio vulgaris</i> 'Miyazaki F	DvMF_2481		[104]
<i>Desulfovibrio vulgaris</i> sp. <i>vulgaris</i> str. <i>Hildenborough</i>	DVU3183		[20,54,97,105-108]
<i>Desulfovibrio gigas</i>	nlr		[22,26,109]
<i>Clostridium acetobutylicum</i> ATCC 824	CAC2450		[110,111]
<i>Nanoarchaeum equitans</i> Kin4-M	NEQ011		[112]

PDB: Protein Data Bank (<http://www.pdb.org/pdb/home/home.do>).

PMID: PubMed unique identifier (<http://www.ncbi.nlm.nih.gov/pubmed>).

combinations of domains makes the previous classification into three classes inappropriate. Indeed, we suggest that the existence of multi-domain SOR indicates new function due to cooperation between domains. As previously proposed, the concept of orthology is more relevant at the level of domains than at the level of whole proteins except for proteins with identical domain architectures [49,50]. We therefore propose a new unambiguous SOR classification based on their domain architectures (sequential order of domains from the N- to the C-terminus [49]). Considering both domain compositions and arrangements, this classification contains seven functionally relevant classes which were precisely described on the website (<http://sorgo.genouest.org/classif.php>, additional file 1 and Table 2). Briefly, the 144 proteins that contain only the active site II (SOR) without other additional domains or cofactors have been classified as Class II-related SOR and correspond to the previous SOR class II [20,22,23,51]. Class III-related SOR correspond to the previous SOR class III proteins which have the active site II and enclose an additional N-terminal region of unknown function [25,35,52]. Class-IV related SOR correspond to very recently new class of methanoferrodoxin [53] which have the active site II and an additional iron sulfur domain. The TAT-SOR have the active site II and include an extra twin-arginine N-terminal signal peptide. The 152 proteins composed of a desulfiredoxin (Dx) domain preceding the SOR unit (formerly Class I [20,21,54-56]) were clustered in a class named Dx-SOR. The 19 proteins that combined a N-terminal helix-turn-helix domain (HTH) before the Dx-SOR module were gathered in a separate class called HTH-Dx-SOR. Finally, 10 SOR proteins that correspond to exceptional domains fusion or that encompass a mutated ncDx domain (frameshift or mutation in the conserved CXXCX15CC metal binding residues) were classified in a disparate class labelled "Atypical-SOR". This class is quite heterogeneous but includes all proteins whose composite or mutated structure might suggest a function different of the three previous classes or, in the case of mutants, a non-functionality due to the loss of key binding sites.

#### SORGOdb website construction

SORGOdb is a relational database built on MySQL and accessed from a PHP web-based interface (phpMyAdmin, Ratschiller, 2000) with additional JavaScript and JQuery functionalities (jQuery JavaScript library released

in 2006 by John Resig). The database runs with the Apache web server version 2.2.3, hosted at the BioGenouest bioinformatics platform (<http://www.genouest.org/>). The sequences, features and annotations were introduced into the database using Python-based scripts.

#### SORGOdb Web interface

SORGOdb includes both documentation and search options. The web interface is composed of two panels (Figure 1).

The navigation menu (on the left) provides access to SORGOdb functions through three modules. (i) Browse: browse SOR proteins according to phylogeny criteria (kingdom, phylum, class and order) or locus tag name. (ii) Search: by organism name query and by sequence similarity through a BlastP form that allows users to enter primary sequences to find similar entries into the SORGOdb database and (iii) Pre-computed Results that include data statistics (organized in three tabs), classes (details about SORGOdb classes and ontology) and useful links (reference, tools and websites). Statistical results about SORGOdb classification were presented in the Classification tab (<http://sorgo.genouest.org/classif-Stat.php>).

The results panel (on the right) provides intermediary selection options and displays SOR record information in a tabular way including organism name, locus tag name, SORGOdb classification and domains architecture. When available, SORGOdb includes a CGView [57] representation of the distribution of SOR and all SOD genes (MnSOD, FeSOD CuZnSOD and NiSOD) [36] in the replicons and a gView [58] map to illustrate the genetic organisation and encoded functions surrounding each SOR (window of 11 genes max.).

#### SORGOdb synopsis and download

Using checkboxes, amino acid sequences and bibliography links can be obtained and synopsis cart can be downloading in .pdf format (Figure 2). Synopsis were created and pre-computed for each SOR (using Python scripts and PHP library FPDF v1.6, <http://www.fpdf.org/>) in order to highlight key findings in a unified manner with all protein information (locus tag, ID, organism name, replicon and genome status), previous (PRODOM, PFAM and CDD) and new (SORGOdb) classification, position in the SORGOdb distance tree, SOR cellular localization prediction using CoBaltDB [59], genomic organisation for SOR and SOD *loci*, synteny viewer, PMID and PDB references. Images were generated using Python scripts from CGview (genomic map), MyDomains (SORGOdb domains representation), CDD, PFAM and PRODOM (database domains illustration), gView (synteny organisation) and from FigTree (for distance tree; <http://tree.bio.ed.ac.uk/software/figtree>).

**Table 2 Classes of SOR in SORGOdb (Number of proteins per classes)**

SOR in SORGOdb	Dx-SOR	SOR	HTH-SOR	Atypical SOR
325	152	144	19	10

A

B SORGO results for Archaeoglobi

Organism Name	Locus Tag	SORGO Classification	Domains
<input type="checkbox"/> Archaeoglobus fulgidus DSM 4304	AF0344	Class II related SOR	
<input checked="" type="checkbox"/> Archaeoglobus fulgidus DSM 4304	AF0833	Dx-SOR	
<input checked="" type="checkbox"/> Archaeoglobus profundus DSM 5631	Arcpr_0633	Class II related SOR	
<input checked="" type="checkbox"/> Ferroglobus placidus DSM 10642	Ferp_1979	Class II related SOR	

INCLUDE FOR EACH CHECKED LOCUS TAG :

- Amino Acid sequence
- Synopsis
- Bibliography

C Amino Acid Sequence in FASTA format

```

>AF0344
MELFQZADNKKKHVIVIEVLAEGGVVVKVSGKEIHPHNTTEHHIAWIELVFQPEGSKFFYVVGRAEFAANGA
SVDGPFVTSYGYTDFVAVFAKAKSKGLTAFSYCNHGLANGAETLSL
>AF0833
MTEVWVYKCMVCGNIVVEVHAGRQLVCCGQPKLMEVKTTEGKKEHVPIEREGNKVYVKGVSVAHPMEEQHY
IENLEVIDDGCVHRKQLKPGDEPKAEFVMSDRVRSARAYCNLHGLWQS
>Arcpr_0633
MLESYVYKERAEGEII SKVETHTPEIECPDVKAGEFPEVRISVTKHPNKLHSIRYVDVFFIEGRAFPVKVA
KVQFFPEYAEAEAVLKLKIQSGKIVALAYCNLGLWENYKEVVE
>Ferp_1979
KSCENELPQCHNSVAGKSVDEMTLEKHKHPVITAPDVKAGEFPEVKVETGVVYKHPNKHGHPVSWIEVYLDTPF
VARFDLQFVMSFVTVKVTATHSHEGKRIKARAFCNLHGYNKEVEVE
    
```

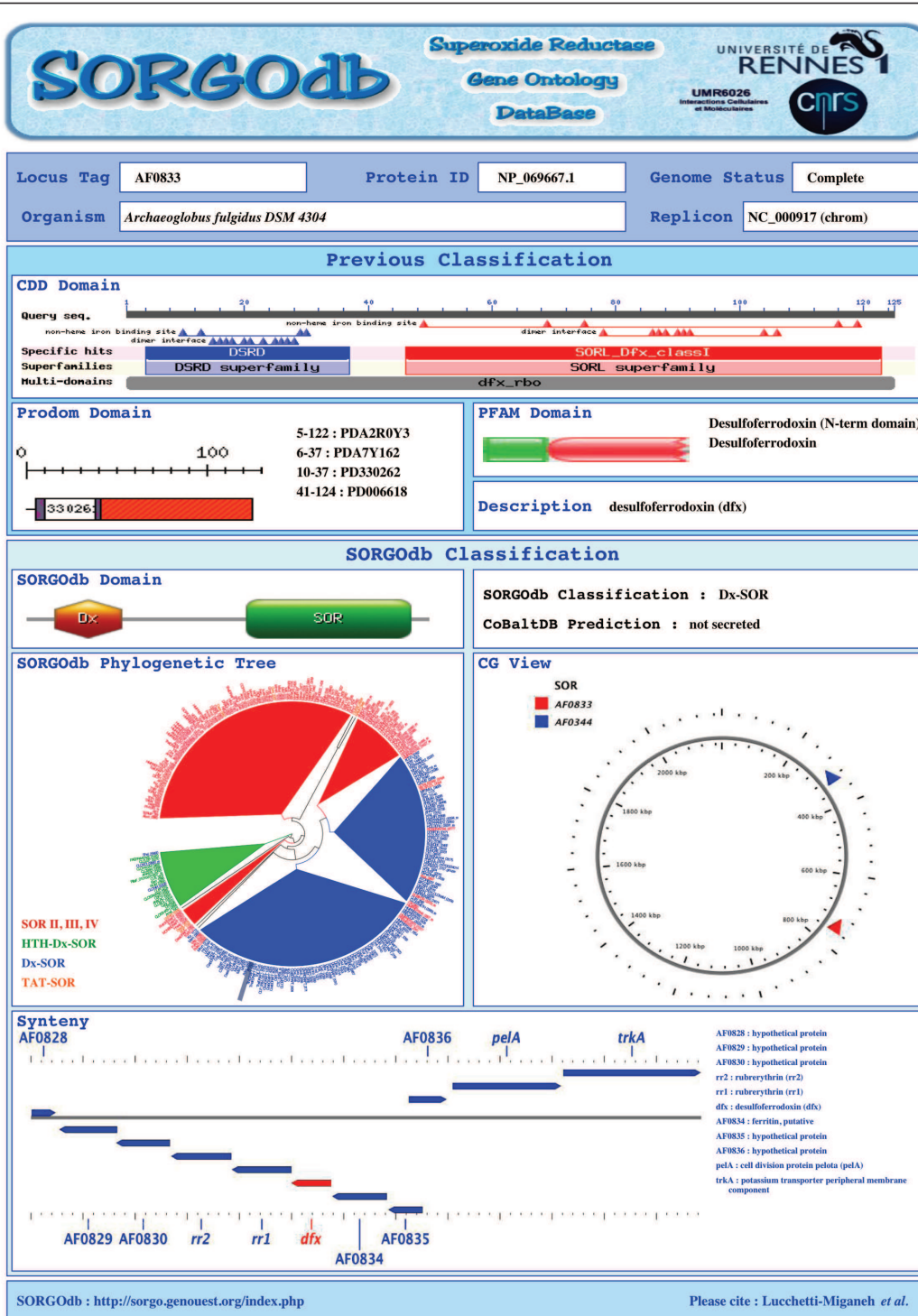
Bibliography Links

LocusTag	PDB link(s)	PMID link(s)
AF0344	No PDB	No PMID
AF0833	No PDB	No PMID
Arcpr_0633	No PDB	No PMID
Ferp_1979	No PDB	No PMID

Download Synopsis

- AF0344.pdf (Archaeoglobus fulgidus DSM 4304)
- AF0833.pdf (Archaeoglobus fulgidus DSM 4304)
- Arcpr\_0633.pdf (Archaeoglobus profundus DSM 5631)
- Ferp\_1979.pdf (Ferroglobus placidus DSM 10642)

**Figure 1 A snapshot of the SORGOdb input interface. (A)** The "Browse By Phylogeny" module allows the selection of organisms with an SOR, using complete phylogeny criteria (kingdom, phylum, class and order). **(B)** The results panel provides intermediary selection options and displays SOR record information in a tabular way including organism name, locus tag name, SORGOdb classification and domain architecture. **(C)** Using checkboxes, amino acid sequences and bibliography links can be obtained and the synopsis can be downloading in .pdf format.

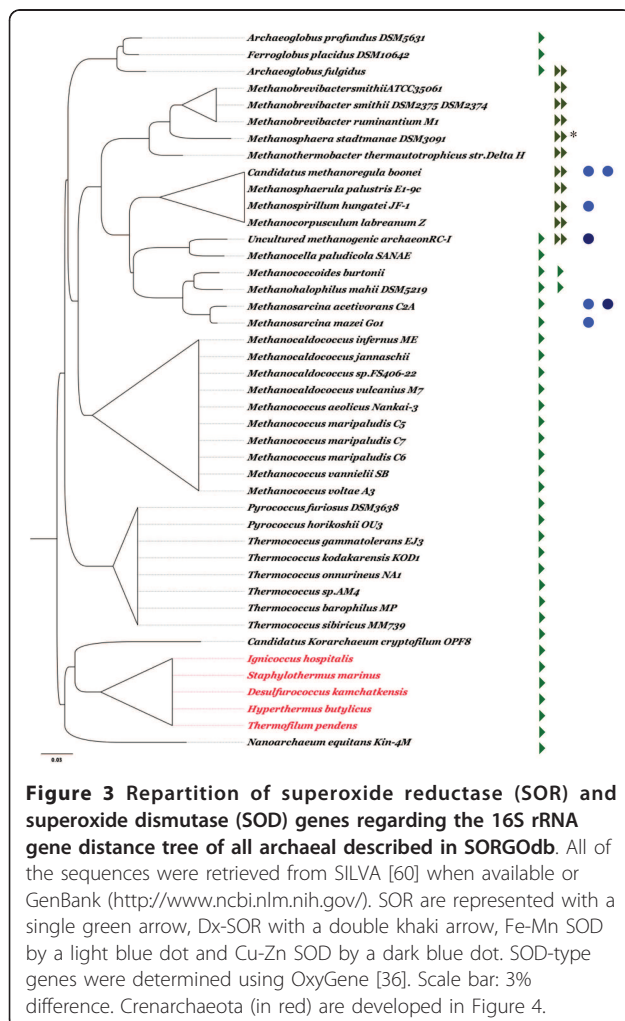


**Figure 2 SORGOdb Synopsis.** For any given protein, all results are summarized in a synopsis which presents results from disparate resources in an unified manner, and includes (i) the previous classification with the SOR description, the domain predictions (ii) the SORGOdb classification with domain representations, the SOR cellular localization prediction, the phylogenetic tree, the position of the sor gene and in some cases the sod gene on the replicon and the local synteny (iii) and bibliography and PDB links when available. This synopsis can be stored as a .pdf file.

### Utility and Discussion

As an example, SORGOdb allows the study of the distribution of genes encoding superoxide reductase across a whole phylum. As a case study, we decided to consider the Archaea as these organisms are considered to be originate from a hyperthermophilic anaerobic common ancestor and were probably already prevalent when the Earth had its primitive anoxic H<sub>2</sub> and CO<sub>2</sub> atmosphere.

Using the “Browse by phylogeny” option of SORGOdb, we collected the names of all Archaea that possess at least one SOR gene in their complete or partial genomes. Then, we generated a 16S-based phylogenetic tree for these organisms, using ClustalW [46] and sequences recovered from the SILVA comprehensible ribosomal RNA databases [60] (<http://www.arb-silva.de/>), clustered by Maximum Likelihood and Neighborhood joining algorithms (Neighborhood joining tree is not shown). This tree was annotated with the class of SOR and the presence of SOD on the genome (Maximum Likelihood Tree; Figure 3).

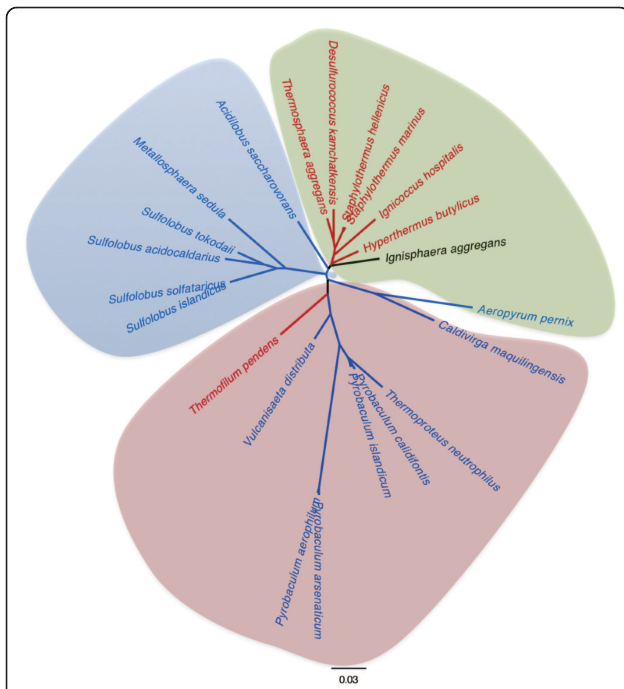


Nanoarchaeota [61] and Korarchaeota [62] are obligately anaerobic sulphur-dependent organisms placed close to the root of the archaeal SSU rRNA tree. Nanoarchaeota is currently known from a single organism *Candidatus Nanoarchaeum equitans*, a hyperthermophilic symbiont that grows on the surface of *Ignicoccus hospitalis* [62,63]. There are currently no representatives of Korarchaeota in pure culture but the genome of *K. cryptophilum*, a very thin filamentous thermophilic heterotroph, has been determined from a sample of Yellowstone National Park Obsidian Pool. Both *C. N. equitans* and *K. cryptophilum* are found together in the 16S tree, in the vicinity of the Crenarchaeota group, and contain genes encoding superoxide reductase with a SOR (centre II) functional domain and do not encode superoxide dismutase genes.

According to 16S rRNA gene sequences, the Crenarchaeota group can be subdivided into three orders, the Thermoproteales, the Sulfolobales and the Desulfurococcales [64]. All Sulfolobales and Thermoproteales genomes studied encode a single SOD, with the single exception of the unique member of the Thermofilaceae family, *Thermofilum pendens*, an anaerobic commensal that encodes a SOR. By contrast, all Desulfurococcales genomes available encode a SOR but not a SOD, except *Aeropyrum pernix* that has the particularity to be strictly aerobic [65] and that encodes an extremely thermostable Mn/Fe superoxide dismutase [66] and *Ignisphaera aggregans*, a novel deep-branching member of the Desulfurococcales lineage of strict anaerobes (as even trace quantities of oxygen inhibited its growth, [67] ) the genome of which carries neither SOR or SOD genes. Other Desulfurococcales studied (Figure 4) have all a gene encoding a centre II monodomain SOR-type enzyme. Interestingly, two recent genomes have been made available since the last update of SORGOdb (May 2010) and both contain annotation for SOR-like genes: Tagg\_0590, described as a Desulfoferrodoxin ferrous iron-binding protein of *Thermosphaera aggregans* DSM 11486 and Shell\_0770 for *Staphylothermus hellenicus* DSM 12710, annotated as a twin-arginine secreted superoxide reductase, by homology with *Geobacter metallireducens* GS-15 Gmet\_2613 SOR. Using the SORGOdb “search by BlastP”, we could confirm that both ORFs are true SOR (ten best e-value from e-59 to e-34) and belong to the SOR-type class. This analysis contradicts the annotation of Shell\_0770 in NCBI as TAT-SOR; the absence of a significant TAT targeting signal in Shell\_0770 was tested and confirmed by TatFind [68] and TatP [69] predictions. The SORGOdb “search by BlastP” tool therefore allows the accuracy of public SOR annotations to be checked and allows suggestions of their possible SORGOdb classification.

Thermococcus and Pyrococcus are obligate anaerobes that live in environments where there is no oxygen and





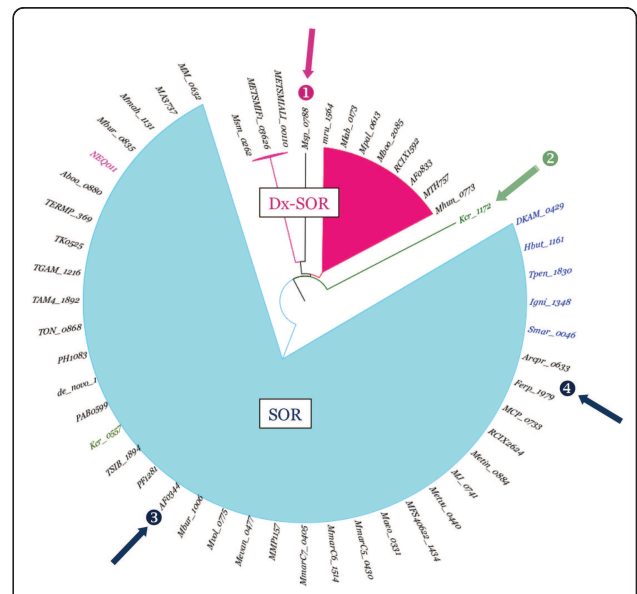
**Figure 4 Repartition of superoxide reductase (SOR) and superoxide dismutase (SOD) genes regarding the 16S rRNA gene distance tree of all Crenarchaeota described in SORGOdb.** All of the sequences were retrieved from SILVA [60] when available or GenBank (<http://www.ncbi.nlm.nih.gov/>). The Thermoproteales are highlighted in red, the Sulfolobales in blue and the Desulfurococcales in green. Organisms having at least one SOR, or one SOD or none of both (any SOD and any SOR) are respectively represented in red, blue and dark.

both produce a SOR-type superoxide reductase that is catalytically active at temperatures below the optimum growth temperature but representing conditions likely corresponding to zones of oxygen exposure [23].

Archaeoglobus is a true archaeal sulphate reducer, reducing  $SO_4^{2-}$  to  $H_2S$  in hot marine sediments. Two complete Archaeoglobus genomes are available, *A. fulgidus* and *A. profundus*. The *A. fulgidus* genome contains one SOR and one Dx-SOR, and the two enzymes have similar kinetics of the superoxide reduction. This raises the question of functional redundancy as Dx-SOR is absent from *A. profundus* and from the related *Ferroglobus placidus*, an iron-oxidising nitrate-reducing species that lives in anoxic (oxygen free) and hot (85°C) environments [70]. The *A. profundus* genome (1.6 Mb) is significantly smaller than those of *A. fulgidus* (2.2 Mb) and *F. placidus* (2.2 Mb). Using the SORGOdb “by organism name search” option, it is easy to compare the genomic locations (GC view map) and the genes contexts (gview synteny map) of the SOR of these three species. This visualization reveals that these genes have different genetic locations and, although the neighbouring genes

encode related functions, the genetic organization and order, are not conserved. Again using the “Browse by phylogeny” option of SORGOdb, we get quickly all archaeal SOR amino acid sequences (using check all then get all amino acid sequence) can be selected and used to cluster by Maximum Likelihood using ClustalW to produce a protein distance-tree (Figure 3). This tree shows the position of each four proteins considered (AF0833, AF0344, Arcpr\_0633 and Ferp\_1979) and indicate that the two *A. fulgidus* SOR (Figure 5, point 3 and 5) are very distant from those of *A. profundus* and *F. placidus*, which by contrast are closely related (Figure 5, point 4). This proximity cannot be linked to the origin of the organisms as *A. fulgidus* and *F. placidus* originate from a shallow marine hydrothermal system at Volcano, Italy [70,71] whereas *A. profundus* was isolated from a deep sea hot vent area (depth: 2000 m) at Guaymas, Mexico [72]. However, based on 16S rRNA gene sequences, indicate that *A. profundus* and *F. placidus* are the most closely related with 96.5% sequence identity.

The protein tree also revealed two interesting phenomena: Msp\_0788 that is a non-canonical Dx-SOR (as the Dx active site is incomplete) that is branched as an out-group close to the entire archaeal Dx-SOR group (Figure 5, point 1). This is consistent with the presumed loss-of-function of Dx of Msp\_0788 being relatively recent. Also, the Kcr\_1172 locus forms a major divergent branch (Figure 5, point 2). Using the “Browse by locus tag” option, Kcr\_1172 is revealed to be a fusion protein with an additional C-terminal module sharing significantly similarities with archaeal proteins annotated



**Figure 5 An evolutionary maximum likelihood tree of archaeal SOR proteins.** The tree shows the repartition of SOR (blue area) and Dx-SOR (pink area) types.

as “hypothetical” or “redoxin domain-containing”. The best-conserved component is a CXXC motif (i.e. cysteines separated by two amino acids), found in many redox proteins for the formation, the isomerization and the reduction of disulphide bonds and for other redox functions [73]. Kcr\_1172 has a new SOR-derived architecture with the presence of two CXXC active sites (in the C-terminal fusion and N-terminal “Dx parts”), separated by the functional SOR centre II. This arrangement is unique and interesting as a combination of two sites CXXC motifs has been shown to be involved in protein disulphide-shuffling in hyperthermophiles [74]. Although the true function of this protein needs to be determined experimentally, we show with this example that SORGOdb can also be used to reveal possible new SOR features.

The distribution of genes encoding SOR and SOD is extremely heterogeneous, both qualitatively and quantitatively, in the group of methanogenic archaea as shown in Figure 3. Thus, for the genus *Methanosarcina*, *Methanosarcina acetivorans* (5.8 Mb) possesses one SOR and two SOD whereas *Methanosarcina mazei* (4.1 Mb) encodes only one SOR. *M. barkeri*, that shares 80% identity with both *M. acetivorans* and *M. mazei* [75], encodes two SOD [36] but no SOR. The presence of these various combinations of oxygen-dependent SOD and SOR genes confirm that methanogens, that are sensitive to oxygen and are rapidly killed by even very low concentrations of O<sub>2</sub>, protect themselves from ROS; however, the factors that influence the presence and evolution of these genes remain unidentified. No clear relationship can be established between oxygen tolerance and the existence of superoxide reductase functions in the genome of microbes. A difficulty is the different connotations of the term ‘anoxia’ as used by geologists, zoologists and microbiologists. Geologists call an environment ‘aerobic’ if the oxygen content exceeds 18%. Zoologists talk about ‘hypoxic’ conditions when referring to oxygen levels that limit respiration (usually less than ca. 50% O<sub>2</sub>). For microbiologists, the so-called ‘Pasteur point’ of switch from aerobic respiration to fermentation is generally less than about 1 per cent of the atmospheric levels of oxygen; microbes, though, are affected by very low levels of oxygen, often much less than 0.1 per cent whereas some “anaerobes” living today are able to tolerate oxygen even at higher levels.

## Conclusions

The SORGOdb server is the first web server that centralizes and provides an interface for information concerning superoxide reductase proteins. SORGOdb provides integrated features: (1) Multiple options for data browsing and searching (2) Complete descriptions of SOR and a new domain-based classification (3) Synthetic and

downloadable synopsis for each locus tag (4) A SOR-homology analysis tool using BlastP similarity searches with the SORGOdb-positive dataset (5) An integrated access to external hyperlinks to various public data sources (notably NCBI GenBank, and Pubmed). SORGOdb is a unique mining tool that can assist researchers with diverse interests to retrieve, visualize and analyse superoxide reductase genes and proteins.

## Availability and requirements

**Database name:** SORGOdb

**Project home page:** <http://sorgo.genouest.org/index.php>

**Operating system(s):** Platform independent, designed for Safari and Firefox browser and not available for Internet Explorer.

**Programming languages:** PHP5 (PHP4 compatible), (X)HTML, CSS2, JavaScript, JQuery, MySQL 5.

## Additional material

**Additional file 1: Distance trees and alignments for each SORGOdb classes and subclasses.** The Dx-SOR (Figure A) and Class II-related SOR (Figure B) trees, based on genetic distances, were constructed using ClustalW and UPGMA algorithm. Clade divisions are illustrated by alternatively pink and yellow highlighted area and sequences selected to represent each clade in the alignment are written in red. Multiple sequence alignment were performed using ClustalW and visualized with Jalview [113,114]. Conserved amino acids are highlighted with different shades of blue considering the degree of identity (most conserved amino acids are coloured in dark blue). These alignments correspond to selected Dx-SOR (Figure C), selected Class II-related SOR (Figure D), all Class III-related SOR (Figure E), all Class IV-related SOR (Figure F), all TAT-SOR (Figure G) and all HTH-Dx-SOR (Figure H). Residues that bind the catalytic center are indicated by a blue asterisk. The amino acid sequences corresponding to SOR which have been biochemically characterized are indicated by a blue arrow. The different SOR domains for each class of SOR, are represented just below multiple sequence alignment.

## List of abbreviations used

Dfx : Desulfoferrodoxin  
Dx : Desulfoferrodoxin  
Nlr : Neelaredoxin  
ROS : Reactive Oxygen Species  
SOD : superoxide dismutase  
SOR : superoxide reductase  
TAT : Twin-arginine translocation

## Acknowledgements

CLM is supported by Agence Nationale de la Recherche and DG by the Ministère de la Recherche. We wish to thank the bioinformatics platform of Biogenouest of Rennes for providing the hosting infrastructure.

## Author details

<sup>1</sup>CNRS UMR 6026, ICM, Equipe Sp@rte, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes, France. <sup>2</sup>EMBL-EBI Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, CB10 1SD, UK.

## Authors' contributions

CLM and FBH jointly carried out the literature survey and designed the study. CLM and FBH retrieved, analyzed, prepared the SOR dataset

(sequence, reference, ontology...) and illustrated the relational database. DT and DG performed scripts for automated data retrieval. CLM developed the original web pages and FBH proposed design improvements. DG and CLM worked together on the PHP code. DG conceived the synopsis computation and performed all debugging activities. CLM and FBH wrote the manuscript. FBH managed the project. GS is the Sp@rte team leader and provides CLM financial support. All authors read and approved the final manuscript.

Received: 15 December 2010 Accepted: 16 May 2011

Published: 16 May 2011

## References

- Holland HD: The oxygenation of the atmosphere and oceans. *Philos Trans R Soc Lond B Biol Sci* 2006, **361(1470)**:903-915.
- Kasting JF: Earth's early atmosphere. *Science* 1993, **259(5097)**:920-926.
- Massey V, Strickland S, Mayhew SG, Howell LG, Engel PC, Matthews RG, Schuman M, Sullivan PA: The production of superoxide anion radicals in the reaction of reduced flavins and flavoproteins with molecular oxygen. *Biochem Biophys Res Commun* 1969, **36(6)**:891-897.
- Imlay JA: Cellular defenses against superoxide and hydrogen peroxide. *Annu Rev Biochem* 2008, **77**:755-776.
- Imlay JA: Pathways of oxidative damage. *Annu Rev Microbiol* 2003, **57**:395-418.
- Kuo CF, Mashino T, Fridovich I: alpha, beta-Dihydroxyisovalerate dehydratase. A superoxide-sensitive enzyme. *J Biol Chem* 1987, **262(10)**:4724-4727.
- Flint DH, Tuminello JF, Emptage MH: The inactivation of Fe-S cluster containing hydro-lyases by superoxide. *J Biol Chem* 1993, **268(30)**:22369-22376.
- Adams MW, Holden JF, Menon AL, Schut GJ, Grunden AM, Hou C, Hutchins AM, Jenney FE Jr, Kim C, Ma K, et al: Key role for sulfur in peptide metabolism and in regulation of three hydrogenases in the hyperthermophilic archaeon *Pyrococcus furiosus*. *J Bacteriol* 2001, **183(2)**:716-724.
- McCord JM, Fridovich I: Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem* 1969, **244(22)**:6049-6055.
- Sturtz LA, Diekert K, Jensen LT, Lill R, Culotta VC: A fraction of yeast Cu,Zn-superoxide dismutase and its metallochaperone, CCS, localize to the intermembrane space of mitochondria. A physiological role for SOD1 in guarding against mitochondrial oxidative damage. *J Biol Chem* 2001, **276(41)**:38084-38089.
- Landis GN, Tower J: Superoxide dismutase evolution and life span regulation. *Mech Ageing Dev* 2005, **126(3)**:365-379.
- Abreu IA, Cabelli DE: Superoxide dismutases-a review of the metal-associated mechanistic variations. *Biochim Biophys Acta* 2010, **1804(2)**:263-274.
- Pilon M, Ravet K, Tapken W: The biogenesis and physiological function of chloroplast superoxide dismutases. *Biochim Biophys Acta* 2010.
- Myouga F, Hosoda C, Umezawa T, Iizumi H, Kurumori T, Motohashi R, Shono Y, Nagata N, Ikeuchi M, Shinozaki K: A heterocomplex of iron superoxide dismutases defends chloroplast nucleoids against oxidative stress and is essential for chloroplast development in *Arabidopsis*. *Plant Cell* 2008, **20(11)**:3148-3162.
- Hassan HM: Microbial superoxide dismutases. *Adv Genet* 1989, **26**:65-97.
- Youn HD, Kim EJ, Roe JH, Hah YC, Kang SO: A novel nickel-containing superoxide dismutase from *Streptomyces* spp. *Biochem J* 1996, **318(Pt 3)**:889-896.
- Youn HD, Youn H, Lee JW, Yim YI, Lee JK, Hah YC, Kang SO: Unique isozymes of superoxide dismutase in *Streptomyces griseus*. *Arch Biochem Biophys* 1996, **334(2)**:341-348.
- Palenik B, Brahamsha B, Larimer FW, Land M, Hauser L, Chain P, Lamerdin J, Regala W, Allen EE, McCarren J, et al: The genome of a motile marine *Synechococcus*. *Nature* 2003, **424(6952)**:1037-1042.
- McCord JM, Keele BB Jr, Fridovich I: An enzyme-based theory of obligate anaerobiosis: the physiological function of superoxide dismutase. *Proc Natl Acad Sci USA* 1971, **68(5)**:1024-1027.
- Moura I, Tavares P, Moura JJ, Ravi N, Huynh BH, Liu MY, LeGall J: Purification and characterization of desulfoferrodoxin. A novel protein from *Desulfovibrio desulfuricans* (ATCC 27774) and from *Desulfovibrio vulgaris* (strain Hildenborough) that contains a distorted rubredoxin center and a mononuclear ferrous center. *J Biol Chem* 1990, **265(35)**:21596-21602.
- Lombard M, Fontecave M, Touati D, Niviere V: Reaction of the desulfoferrodoxin from *Desulfovibrio baarsii* with superoxide anion. Evidence for a superoxide reductase activity. *J Biol Chem* 2000, **275(1)**:115-121.
- Chen L, Sharma P, Le Gall J, Mariano AM, Teixeira M, Xavier AV: A blue non-heme iron protein from *Desulfovibrio gigas*. *Eur J Biochem* 1994, **226(2)**:613-618.
- Jenney FE Jr, Verhagen MF, Cui X, Adams MW: Anaerobic microbes: oxygen detoxification without superoxide dismutase. *Science* 1999, **286(5438)**:306-309.
- Pianzola MJ, Soubes M, Touati D: Overproduction of the rbo gene product from *Desulfovibrio* species suppresses all deleterious effects of lack of superoxide dismutase in *Escherichia coli*. *J Bacteriol* 1996, **178(23)**:6736-6742.
- Lombard M, Touati D, Fontecave M, Niviere V: Superoxide reductase as a unique defense system against superoxide stress in the microaerophile *Treponema pallidum*. *J Biol Chem* 2000, **275(35)**:27021-27026.
- Silva G, LeGall J, Xavier AV, Teixeira M, Rodrigues-Pousada C: Molecular characterization of *Desulfovibrio gigas* neelaredoxin, a protein involved in oxygen detoxification in anaerobes. *J Bacteriol* 2001, **183(15)**:4413-4420.
- Liochev SI, Fridovich I: A mechanism for complementation of the sodA sodB defect in *Escherichia coli* by overproduction of the rbo gene product (desulfoferrodoxin) from *Desulfovibrio baarsii*. *J Biol Chem* 1997, **272(41)**:25573-25575.
- Tulipan DJ, Eaton RG, Eberhart RE: The Darrach procedure definded: technique redefined and long-term follow-up. *J Hand Surg Am* 1991, **16(3)**:438-444.
- Clay MD, Jenney FE Jr, Hagedoorn PL, George GN, Adams MW, Johnson MK: Spectroscopic studies of *Pyrococcus furiosus* superoxide reductase: implications for active-site structures and the catalytic mechanism. *J Am Chem Soc* 2002, **124(5)**:788-805.
- Yeh AP, Hu Y, Jenney FE Jr, Adams MW, Rees DC: Structures of the superoxide reductase from *Pyrococcus furiosus* in the oxidized and reduced states. *Biochemistry* 2000, **39(10)**:2499-2508.
- Coelho AV, Matias PM, Fulop V, Thompson A, Gonzalez A, Carrondo MA: Desulfoferrodoxin structure determined by MAD phasing and refinement to 1.9-Å resolution reveals a unique combination of a tetrahedral FeS4 centre with a square pyramidal FeS4 centre. *J Biol Inorg Chem* 1997, **2(6)**:680-689.
- Archer M, Huber R, Tavares P, Moura I, Moura JJ, Carrondo MA, Sieker LC, LeGall J, Romao MJ: Crystal structure of desulfoferrodoxin from *Desulfovibrio gigas* determined at 1.8 Å resolution: a novel non-heme iron protein structure. *J Mol Biol* 1995, **251(5)**:690-702.
- Kurtz DM Jr, Coulter ED: The mechanism(s) of superoxide reduction by superoxide reductases in vitro and in vivo. *J Biol Inorg Chem* 2002, **7(6)**:653-658.
- Pereira SA, Tavares P, Folgosa F, Almeida RM, Moura I, Moura JGG: European Journal of Inorganic Chemistry. *European Journal of Inorganic Chemistry* 2007, **2007(18)**:2569-2581.
- Jovanovic T, Ascenso C, Hazlett KR, Sikkink R, Krebs C, Litwiller R, Benson LM, Moura I, Moura JJ, Radolf JD, et al: Neelaredoxin, an iron-binding protein from the syphilis spirochete, *Treponema pallidum*, is a superoxide reductase. *J Biol Chem* 2000, **275(37)**:28439-28448.
- Thybert D, Avner S, Lucchetti-Miganeh C, Cheron A, Barloy-Hubler F: OxyGene: an innovative platform for investigating oxidative-response genes in whole prokaryotic genomes. *BMC Genomics* 2008, **9**:637.
- Brioukhanov AL, Netrusov AI: Catalase and superoxide dismutase: distribution, properties, and physiological role in cells of strict anaerobes. *Biochemistry (Mosc)* 2004, **69(9)**:949-962.
- Tally FP, Goldin BR, Jacobus NV, Gorbach SL: Superoxide dismutase in anaerobic bacteria of clinical significance. *Infect Immun* 1977, **16(1)**:20-25.
- Rusnak F, Ascenso C, Moura I, Moura JJ: Superoxide reductase activities of neelaredoxin and desulfoferrodoxin metalloproteins. *Methods Enzymol* 2002, **349**:243-258.
- Niviere V, Fontecave M: Discovery of superoxide reductase: an historical perspective. *J Biol Inorg Chem* 2004, **9(2)**:119-123.
- Pinto AF, Rodrigues JV, Teixeira M: Reductive elimination of superoxide: Structure and mechanism of superoxide reductases. *Biochim Biophys Acta* 2010, **1804(2)**:285-297.

42. Skovgaard M, Jensen LJ, Brunak S, Ussery D, Krogh A: **On the total number of genes and their length distribution in complete microbial genomes.** *Trends Genet* 2001, **17(8)**:425-428.
43. Dolla A, Fournier M, Dermoun Z: **Oxygen defense in sulfate-reducing bacteria.** *J Biotechnol* 2006, **126(1)**:87-100.
44. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: **Basic local alignment search tool.** *J Mol Biol* 1990, **215(3)**:403-410.
45. Gertz EM, Yu YK, Agarwala R, Schaffer AA, Altschul SF: **Composition-based statistics and translated nucleotide searches: improving the TBLASTN module of BLAST.** *BMC Biol* 2006, **4**:41.
46. Thompson JD, Higgins DG, Gibson TJ: **CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice.** *Nucleic Acids Res* 1994, **22(22)**:4673-4680.
47. Higgins DG, Thompson JD, Gibson TJ: **Using CLUSTAL for multiple sequence alignments.** *Methods Enzymol* 1996, **266**:383-402.
48. Edgar RC: **MUSCLE: a multiple sequence alignment method with reduced time and space complexity.** *BMC Bioinformatics* 2004, **5**:113.
49. Koonin EV, Wolf YI, Karev GP: **The structure of the protein universe and genome evolution.** *Nature* 2002, **420(6912)**:218-223.
50. Ponting CP, Russell RR: **The natural history of protein domains.** *Annu Rev Biophys Biomol Struct* 2002, **31**:45-71.
51. Abreu IA, Saraiva LM, Carita J, Huber H, Stetter KO, Cabelli D, Teixeira M: **Oxygen detoxification in the strict anaerobic archaeon *Archaeoglobus fulgidus*: superoxide scavenging by neelaredoxin.** *Mol Microbiol* 2000, **38(2)**:322-334.
52. Mathe C, Niviere V, Houee-Levin C, Mattioli TA: **Fe(3+)-eta(2)-peroxo species in superoxide reductase from *Treponema pallidum*. Comparison with *Desulfoarculus baarsii*.** *Biophys Chem* 2006, **119(1)**:38-48.
53. Kratzer C, Welte C, Dorner K, Friedrich T, Deppenmeier U: **Methanoferrodoxin represents a new class of superoxide reductase containing an iron-sulfur cluster.** *FEBS J* 2011, **278(3)**:442-451.
54. Coulter ED, Kurtz DM Jr: **A role for rubredoxin in oxidative stress protection in *Desulfovibrio vulgaris*: catalytic electron transfer to rubrerythrin and two-iron superoxide reductase.** *Arch Biochem Biophys* 2001, **394(1)**:76-86.
55. Rodrigues JV, Saraiva LM, Abreu IA, Teixeira M, Cabelli DE: **Superoxide reduction by *Archaeoglobus fulgidus* desulfoferrodoxin: comparison with neelaredoxin.** *J Biol Inorg Chem* 2007, **12(2)**:248-256.
56. Coelho AV, Matias PM, Carrondo MA, Tavares P, Moura JJ, Moura I, Fulop V, Hajdu J, Le Gall J: **Preliminary crystallographic analysis of the oxidized form of a two mono-nuclear iron centres protein from *Desulfovibrio desulfuricans* ATCC 27774.** *Protein Sci* 1996, **5(6)**:1189-1191.
57. Stothard P, Wishart DS: **Circular genome visualization and exploration using CGView.** *Bioinformatics* 2005, **21(4)**:537-539.
58. Petkau A, Stuart-Edwards M, Stothard P, Van Domselaar G: **Interactive Microbial Genome Visualization with GView.** *Bioinformatics* 2010.
59. Goudenege D, Avner S, Lucchetti-Miganeh C, Barloy-Hubler F: **CoBaltDB: Complete bacterial and archaeal orfeomes subcellular localization database and associated resources.** *BMC Microbiol* 2010, **10**:88.
60. Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, Glockner FO: **SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB.** *Nucleic Acids Res* 2007, **35(21)**:7188-7196.
61. Barns SM, Delwiche CF, Palmer JD, Dawson SC, Hershberger KL, Pace NR: **Phylogenetic perspective on microbial life in hydrothermal ecosystems, past and present.** *Ciba Found Symp* 1996, **202**:24-32, discussion 32-29.
62. Huber H, Hohn MJ, Rachel R, Fuchs T, Wimmer VC, Stetter KO: **A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont.** *Nature* 2002, **417(6884)**:63-67.
63. Paper W, Jahn U, Hohn MJ, Kronner M, Nather DJ, Burghardt T, Rachel R, Stetter KO, Huber H: ***Ignicoccus hospitalis* sp. nov., the host of 'Nanoarchaeum equitans'.** *Int J Syst Evol Microbiol* 2007, **57(Pt 4)**:803-808.
64. Burggraf S, Huber H, Stetter KO: **Reclassification of the crenarchaeal orders and families in accordance with 16S rRNA sequence data.** *Int J Syst Bacteriol* 1997, **47(3)**:657-660.
65. Kawarabayasi Y, Hino Y, Horikawa H, Yamazaki S, Haikawa Y, Jin-no K, Takahashi M, Sekine M, Baba S, Ankai A, et al: **Complete genome sequence of an aerobic hyper-thermophilic crenarchaeon, *Aeropyrum pernix* K1.** *DNA Res* 1999, **6(2)**:83-101, 145-152.
66. Lee HJ, Kwon HW, Koh JU, Lee DK, Moon JY, Kong KH: **An efficient method for the expression and reconstitution of thermostable Mn/Fe superoxide dismutase from *Aeropyrum pernix* K1.** *J Microbiol Biotechnol* 2010, **20(4)**:727-731.
67. Niederberger TD, Gotz DK, McDonald IR, Ronimus RS, Morgan HW: ***Ignisphaera aggregans* gen. nov., sp. nov., a novel hyperthermophilic crenarchaeote isolated from hot springs in Rotorua and Tokaanu, New Zealand.** *Int J Syst Evol Microbiol* 2006, **56(Pt 5)**:965-971.
68. Rose RW, Bruser T, Kissinger JC, Pohlschroder M: **Adaptation of protein secretion to extremely high-salt conditions by extensive use of the twin-arginine translocation pathway.** *Mol Microbiol* 2002, **45(4)**:943-950.
69. Bendtsen JD, Nielsen H, Widdick D, Palmer T, Brunak S: **Prediction of twin-arginine signal peptides.** *BMC Bioinformatics* 2005, **6**:167.
70. Hafenbradl D, Keller M, Dirmeier R, Rachel R, Rossnagel P, Burggraf S, Huber H, Stetter KO: ***Ferroglobus placidus* gen. nov., sp. nov., A novel hyperthermophilic archaeum that oxidizes Fe<sup>2+</sup> at neutral pH under anoxic conditions.** *Arch Microbiol* 1996, **166(5)**:308-314.
71. Klenk HP, Clayton RA, Tomb JF, White O, Nelson KE, Ketchum KA, Dodson RJ, Gwinn M, Hickey EK, Peterson JD, et al: **The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*.** *Nature* 1997, **390(6658)**:364-370.
72. Burggraf S, Jannasch HW, Nicolaus B, Stetter KO: ***Archaeoglobus profundus* sp. nov., represents a new species within the sulfate-reducing archaeobacteria.** *Syst Appl Microbiol* 1990, **13**:24-28.
73. Fomenko DE, Gladyshev VN: **Identity and functions of CxxC-derived motifs.** *Biochemistry* 2003, **42(38)**:11214-11225.
74. Ladenstein R, Ren B: **Reconsideration of an early dogma, saying "there is no evidence for disulfide bonds in proteins from archaea".** *Extremophiles* 2008, **12(1)**:29-38.
75. Maeder DL, Anderson I, Brettin TS, Bruce DC, Gilna P, Han CS, Lapidus A, Metcalf WW, Saunders E, Tapia R, et al: **The *Methanosarcina barkeri* genome: comparative analysis with *Methanosarcina acetivorans* and *Methanosarcina mazei* reveals extensive rearrangement within methanosarcinal genomes.** *J Bacteriol* 2006, **188(22)**:7922-7931.
76. Devreese B, Tavares P, Lamprea J, Van Damme N, Le Gall J, Moura JJ, Van Beeumen J, Moura I: **Primary structure of desulfoferrodoxin from *Desulfovibrio desulfuricans* ATCC 27774, a new class of non-heme iron proteins.** *FEBS Lett* 1996, **385(3)**:138-142.
77. Tavares P, Ravi N, Moura JJ, LeGall J, Huang YH, Crouse BR, Johnson MK, Huynh BH, Moura I: **Spectroscopic properties of desulfoferrodoxin from *Desulfovibrio desulfuricans* (ATCC 27774).** *J Biol Chem* 1994, **269(14)**:10504-10510.
78. Romao CV, Liu MY, Le Gall J, Gomes CM, Braga V, Pacheco I, Xavier AV, Teixeira M: **The superoxide dismutase activity of desulfoferrodoxin from *Desulfovibrio desulfuricans* ATCC 27774.** *Eur J Biochem* 1999, **261(2)**:438-443.
79. Adam V, Royant A, Niviere V, Molina-Heredia FP, Bourgeois D: **Structure of superoxide reductase bound to ferrocyanide and active site expansion upon X-ray-induced photo-reduction.** *Structure* 2004, **12(9)**:1729-1740.
80. Katona G, Carpentier P, Niviere V, Amara P, Adam V, Ohana J, Tsanov N, Bourgeois D: **Raman-assisted crystallography reveals end-on peroxide intermediates in a nonheme iron enzyme.** *Science* 2007, **316(5823)**:449-453.
81. Niviere V, Asso M, Weill CO, Lombard M, Guigliarelli B, Favaudon V, Houee-Levin C: **Superoxide reductase from *Desulfoarculus baarsii*: identification of protonation steps in the enzymatic mechanism.** *Biochemistry* 2004, **43(3)**:808-818.
82. Mathe C, Mattioli TA, Horner O, Lombard M, Latour JM, Fontecave M, Niviere V: **Identification of iron(III) peroxo species in the active site of the superoxide reductase SOR from *Desulfoarculus baarsii*.** *J Am Chem Soc* 2002, **124(18)**:4966-4967.
83. Mathe C, Weill CO, Mattioli TA, Berthomieu C, Houee-Levin C, Tremey E, Niviere V: **Assessing the role of the active-site cysteine ligand in the superoxide reductase from *Desulfoarculus baarsii*.** *J Biol Chem* 2007, **282(30)**:22207-22216.
84. Mathe C, Niviere V, Mattioli TA: **Fe<sup>3+</sup>-hydroxide ligation in the superoxide reductase from *Desulfoarculus baarsii* is associated with pH dependent spectral changes.** *J Am Chem Soc* 2005, **127(47)**:16436-16441.
85. Horner O, Mouesca JM, Oddou JL, Jeandey C, Niviere V, Mattioli TA, Mathe C, Fontecave M, Maldivi P, Bonville P, et al: **Mossbauer characterization of an unusual high-spin side-on peroxo-Fe<sup>3+</sup> species in the active site of superoxide reductase from *Desulfoarculus Baarsii*.**

- Density functional calculations on related models. *Biochemistry* 2004, **43**(27):8815-8825.
86. Berthomieu C, Dupeyrat F, Fontecave M, Vermeglio A, Niviere V: Redox-dependent structural changes in the superoxide reductase from *Desulfoarculus baarsii* and *Treponema pallidum*: a FTIR study. *Biochemistry* 2002, **41**(32):10360-10368.
87. Bonnot F, Houee-Levin C, Favaudon V, Niviere V: Photochemical processes observed during the reaction of superoxide reductase from *Desulfoarculus baarsii* with superoxide: re-evaluation of the reaction mechanism. *Biochim Biophys Acta* 2010, **1804**(4):762-767.
88. Clay MD, Jenney FE Jr, Noh HJ, Hagedoorn PL, Adams MW, Johnson MK: Resonance Raman characterization of the mononuclear iron active-site vibrations and putative electron transport pathways in *Pyrococcus furiosus* superoxide reductase. *Biochemistry* 2002, **41**(31):9833-9841.
89. Grunden AM, Jenney FE Jr, Ma K, Ji M, Weinberg MV, Adams MW: In vitro reconstitution of an NADPH-dependent superoxide reduction pathway from *Pyrococcus furiosus*. *Appl Environ Microbiol* 2005, **71**(3):1522-1530.
90. Clay MD, Coper CA, Jenney FE Jr, Adams MW, Johnson MK: Nitric oxide binding at the mononuclear active site of reduced *Pyrococcus furiosus* superoxide reductase. *Proc Natl Acad Sci USA* 2003, **100**(7):3796-3801.
91. Im YJ, Ji M, Lee A, Killens R, Grunden AM, Boss WF: Expression of *Pyrococcus furiosus* superoxide reductase in *Arabidopsis* enhances heat tolerance. *Plant Physiol* 2009, **151**(2):893-904.
92. Santos-Silva T, Trincao J, Carvalho AL, Bonifacio C, Auchere F, Raleiras P, Moura I, Moura JJ, Romao MJ: The first crystal structure of class III superoxide reductase from *Treponema pallidum*. *J Biol Inorg Chem* 2006, **11**(5):548-558.
93. Santos-Silva T, Trincao J, Carvalho AL, Bonifacio C, Auchere F, Moura I, Moura JJ, Romao MJ: Superoxide reductase from the syphilis spirochete *Treponema pallidum*: crystallization and structure determination using soft X-rays. *Acta Crystallogr Sect F Struct Biol Cryst Commun* 2005, **61**(Pt 11):967-970.
94. Niviere V, Lombard M, Fontecave M, Houee-Levin C: Pulse radiolysis studies on superoxide reductase from *Treponema pallidum*. *FEBS Lett* 2001, **497**(2-3):171-173.
95. Auchere F, Sikkink R, Cordas C, Raleiras P, Tavares P, Moura I, Moura JJ: Overexpression and purification of *Treponema pallidum* rubredoxin; kinetic evidence for a superoxide-mediated electron transfer with the superoxide reductase neelaredoxin. *J Biol Inorg Chem* 2004, **9**(7):839-849.
96. Hazlett KR, Cox DL, Sikkink RA, Auch'ere F, Rusnak F, Radolf JD: Contribution of neelaredoxin to oxygen tolerance by *Treponema pallidum*. *Methods Enzymol* 2002, **353**:140-156.
97. Auchere F, Raleiras P, Benson L, Venyaminov SY, Tavares P, Moura JJ, Moura I, Rusnak F: Formation of a stable cyano-bridged dinuclear iron cluster following oxidation of the superoxide reductases from *Treponema pallidum* and *Desulfovibrio vulgaris* with  $K(3)Fe(CN)(6)$ . *Inorg Chem* 2003, **42**(4):938-940.
98. Lombard M, Houee-Levin C, Touati D, Fontecave M, Niviere V: Superoxide reductase from *Desulfoarculus baarsii*: reaction mechanism and role of glutamate 47 and lysine 48 in catalysis. *Biochemistry* 2001, **40**(16):5032-5040.
99. Niviere V, Lombard M: Superoxide reductase from *Desulfoarculus baarsii*. *Methods Enzymol* 2002, **349**:123-129.
100. Bandejas TM, Romao CV, Rodrigues JV, Teixeira M, Matias PM: Purification, crystallization and X-ray crystallographic analysis of *Archaeoglobus fulgidus* neelaredoxin. *Acta Crystallogr Sect F Struct Biol Cryst Commun* 2010, **66**(Pt 3):316-319.
101. Rodrigues JV, Abreu IA, Cabelli D, Teixeira M: Superoxide reduction mechanism of *Archaeoglobus fulgidus* one-iron superoxide reductase. *Biochemistry* 2006, **45**(30):9266-9278.
102. Todorovic S, Rodrigues JV, Pinto AF, Thomsen C, Hildebrandt P, Teixeira M, Murgida DH: Resonance Raman study of the superoxide reductase from *Archaeoglobus fulgidus*, E12 mutants and a 'natural variant'. *Phys Chem Chem Phys* 2009, **11**(11):1809-1815.
103. Abreu IA, Saraiva LM, Soares CM, Teixeira M, Cabelli DE: The mechanism of superoxide scavenging by *Archaeoglobus fulgidus* neelaredoxin. *J Biol Chem* 2001, **276**(42):38995-39001.
104. Kitamura M, Koshino Y, Kamikawa Y, Kohno K, Kojima S, Miura K, Sagara T, Akutsu H, Kumagai I, Nakaya T: Cloning and expression of the rubredoxin gene from *Desulfovibrio vulgaris* (Miyazaki F)—comparison of the primary structure of desulfoferrodoxin. *Biochim Biophys Acta* 1997, **1351**(1-2):239-247.
105. Huang VW, Emerson JP, Kurtz DM Jr: Reaction of *Desulfovibrio vulgaris* two-iron superoxide reductase with superoxide: insights from stopped-flow spectrophotometry. *Biochemistry* 2007, **46**(40):11342-11351.
106. Wildschut JD, Lang RM, Voordouw JK, Voordouw G: Rubredoxin:oxygen oxidoreductase enhances survival of *Desulfovibrio vulgaris* hildenborough under microaerophilic conditions. *J Bacteriol* 2006, **188**(17):6253-6260.
107. Clay MD, Emerson JP, Coulter ED, Kurtz DM Jr, Johnson MK: Spectroscopic characterization of the  $[Fe(His)(4)(Cys)]$  site in 2Fe-superoxide reductase from *Desulfovibrio vulgaris*. *J Biol Inorg Chem* 2003, **8**(6):671-682.
108. Emerson JP, Coulter ED, Cabelli DE, Phillips RS, Kurtz DM Jr: Kinetics and mechanism of superoxide reduction by two-iron superoxide reductase from *Desulfovibrio vulgaris*. *Biochemistry* 2002, **41**(13):4348-4357.
109. Silva G, Oliveira S, Gomes CM, Pacheco I, Liu MY, Xavier AV, Teixeira M, Legall J, Rodrigues-pousada C: *Desulfovibrio gigas* neelaredoxin. A novel superoxide dismutase integrated in a putative oxygen sensory operon of an anaerobe. *Eur J Biochem* 1999, **259**(1-2):235-243.
110. Riebe O, Fischer RJ, Bahl H: *Desulfoferrodoxin* of *Clostridium acetobutylicum* functions as a superoxide reductase. *FEBS Lett* 2007, **581**(29):5605-5610.
111. Kawasaki S, Sakai Y, Takahashi T, Suzuki I, Niimura Y: O<sub>2</sub> and reactive oxygen species detoxification complex, composed of O<sub>2</sub>-responsive NADH:rubredoxin oxidoreductase-flavoprotein A2-desulfoferrodoxin operon enzymes, rubperoxin, and rubredoxin, in *Clostridium acetobutylicum*. *Appl Environ Microbiol* 2009, **75**(4):1021-1029.
112. Rodrigues JV, Victor BL, Huber H, Saraiva LM, Soares CM, Cabelli DE, Teixeira M: Superoxide reduction by *Nanoarchaeum equitans* neelaredoxin, an enzyme lacking the highly conserved glutamate iron ligand. *J Biol Inorg Chem* 2008, **13**(2):219-228.
113. Clamp M, Cuff J, Searle SM, Barton GJ: The Jalview Java alignment editor. *Bioinformatics* 2004, **20**(3):426-427.
114. Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ: Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics* 2009, **25**(9):1189-1191.

doi:10.1186/1471-2180-11-105

Cite this article as: Lucchetti-Miganeh et al.: SORGOdb: Superoxide Reductase Gene Ontology curated DataBase. *BMC Microbiology* 2011 11:105.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
www.biomedcentral.com/submit

