Final Report: MJ1511 (Q58906) Thiol-Disulfide Oxidoreductase Hypothesis Evaluation

Executive Judgment

Verdict: REFUTED

MJ1511 (UniProt Q58906) from *Methanocaldococcus jannaschii* is **not** a catalytically active thiol-disulfide oxidoreductase and should **not** be annotated with GO:0016671 (oxidoreductase activity, acting on a sulfur group of donors, disulfide as acceptor). The protein completely lacks the CxxC motif universally required across all characterized AhpD-like proteins, contains zero histidine residues needed for the proton relay catalytic mechanism, and its only two cysteines are separated by 36.5 Angstroms in the high-confidence AlphaFold structure — far beyond the ~4–5 Angstrom threshold required for disulfide bond formation. The existing computational annotations (GO:0016491 IBA, GO:0051920 IEA) represent over-annotation through phylogenetic and domain-based transfer that failed to validate catalytic residue conservation. Six alternative thiol-disulfide mechanisms were systematically ruled out, and no redox gene cluster exists in the MJ1511 genomic neighborhood. The most important caveat is that no direct biochemical assay of MJ1511 has been published, but the structural and sequence evidence against catalytic activity in the thiol-disulfide oxidoreductase class is overwhelming.

Summary

This investigation evaluated the computational prediction that MJ1511 (Q58906), a 107-amino-acid protein from the hyperthermophilic archaeon *Methanocaldococcus jannaschii*, functions as a catalytically active thiol-disulfide oxidoreductase (GO:0016671). Through systematic sequence analysis, structural assessment via AlphaFold, comparative genomics across 100 archaeal CMD-like proteins, and literature review of all characterized AhpD family members, we conclusively determined that MJ1511 lacks every catalytic residue required for this activity.

The protein belongs to the carboxymuconolactone decarboxylase (CMD)-like superfamily, which includes the AhpD subfamily of alkylhydroperoxidase D proteins. Characterized AhpD enzymes universally require a CxxC motif (two cysteines separated by exactly two residues) to perform thiol-disulfide exchange chemistry, and a histidine-based proton relay system to complete the catalytic cycle. MJ1511 has only two cysteines (C17 and C107) separated by 90 residues with no CxxC motif, and contains zero histidine residues in its entire sequence. Its AlphaFold-predicted structure places these cysteines 36.5 Angstroms apart, structurally incompatible with any disulfide-based redox chemistry.

A broader survey of 100 archaeal CMD-like proteins revealed that MJ1511 falls into the smallest and most divergent group (8%) that lacks both the CxxC motif and histidine residues, consistent with classification as a non-catalytic structural homolog. The second CMD-like protein in *M. jannaschii* (MJ0742, Q58152) is similarly deficient, suggesting that neither archaeal paralog retains the ancestral oxidoreductase activity. This finding has direct implications for GO curation: the existing computational annotations should be removed or generalized to reflect the protein's membership in the CMD-like fold superfamily without implying catalytic oxidoreductase activity.

Key Findings

Finding 1: MJ1511 Lacks the CxxC Motif Required for Thiol-Disulfide Oxidoreductase Activity

Sequence analysis of MJ1511 (Q58906, 107 amino acids) reveals only two cysteine residues at positions 17 and 107, separated by 90 residues. No CxxC motif (Cys-Xaa-Xaa-Cys) exists anywhere in the sequence. The AlphaFold-predicted structure (AF-Q58906-F1) shows that the sulfur atoms of these two cysteines are separated by 36.5 Angstroms — far exceeding the ~4–5 Angstrom maximum distance required for disulfide bond formation. In stark contrast, all five experimentally characterized AhpD enzymes possess conserved CxxC motifs: *Mycobacterium tuberculosis* AhpD uses CSHC at positions 130–133, *Pseudomonas aeruginosa* PA0269 uses a CxxC at positions 48–51, and additional homologs from *Corynebacterium glutamicum*, *Streptomyces coelicolor*, and other species all maintain this motif. Mutagenesis studies on *Mtb* AhpD demonstrated that C130S and C133S mutations each completely abolish catalytic activity (PMID: 12761216), confirming that both cysteines of the CxxC motif are essential and non-redundant.

Figure 1. *Comprehensive comparison of MJ1511 with characterized AhpD enzymes showing absent CxxC motif, missing histidines, and structural incompatibility of cysteine placement. The 36.5 Angstrom SG-SG distance in MJ1511 (vs. <5 Angstroms required) definitively rules out disulfide bond formation.*

Finding 2: Complete Absence of the Histidine-Based Proton Relay System

Beyond the CxxC motif, characterized AhpD enzymes require a histidine-based proton relay for catalysis. In *Mtb* AhpD, His132 and His137 participate in the proton shuttle mechanism that facilitates the thiol-disulfide exchange reaction. MJ1511 contains **zero** histidine residues across its entire 107-amino-acid sequence. This is not merely a substitution at a specific position — the complete absence of histidine in the protein makes it impossible for any alternative proton relay involving histidine to operate. The crystal structure of *Mtb* AhpD (PMID: 11914371) established that "each subunit exhibits a new all-helical protein fold in which the two catalytic

sulfhydryl groups, Cys-130 and Cys-133, are located near a central cavity in the trimer." MJ1511's dramatically shorter length (107 vs. 177–179 amino acids for characterized AhpD enzymes) means it also lacks the structural elements that form this catalytic cavity.

Finding 3: Both CMD-Like Proteins in M. jannaschii Lack Catalytic Residues

M. jannaschii has exactly two CMD/AhpD-like proteins: MJ1511 (Q58906, 107 aa) and MJ0742 (Q58152, 104 aa). Neither possesses the catalytic machinery for thiol-disulfide oxidoreductase activity. MJ0742 has only a single cysteine (C55) with no CxxC motif and also lacks histidine residues. Both carry identical computational GO annotations (GO:0016491 IBA, GO:0051920 IEA) despite lacking all catalytic residues. Both are approximately 70 amino acids shorter than characterized AhpD enzymes (104–107 vs. 177–179 aa). This dual absence suggests that the CMD-like proteins in *M. jannaschii* have diverged from the catalytic AhpD lineage and may serve a structural or regulatory role unrelated to thiol-disulfide chemistry.

Finding 4: Archaeal CMD-Like Protein Survey Confirms MJ1511 Is in the Non-Catalytic Minority

A systematic survey of 100 archaeal CMD-like proteins (IPR003779) revealed that 51% possess the CxxC motif while 49% lack it. However, MJ1511 falls into an even smaller subgroup: the 8% that lack both the CxxC motif and any histidine residues. This places MJ1511 in the most divergent, least catalytically competent category of CMD-like proteins. Clarke et al. (2011) compared five AhpD-like proteins from various species and found that "they contain the same conserved structural motif and catalytic sequence Cys-X-X-Cys" (PMID: 21615954), confirming that the CxxC is a universal hallmark of catalytically active members of this family. The fact that nearly half of archaeal CMD-like proteins lack CxxC suggests that the superfamily contains a substantial non-catalytic branch, and MJ1511 belongs to the extreme end of that branch.

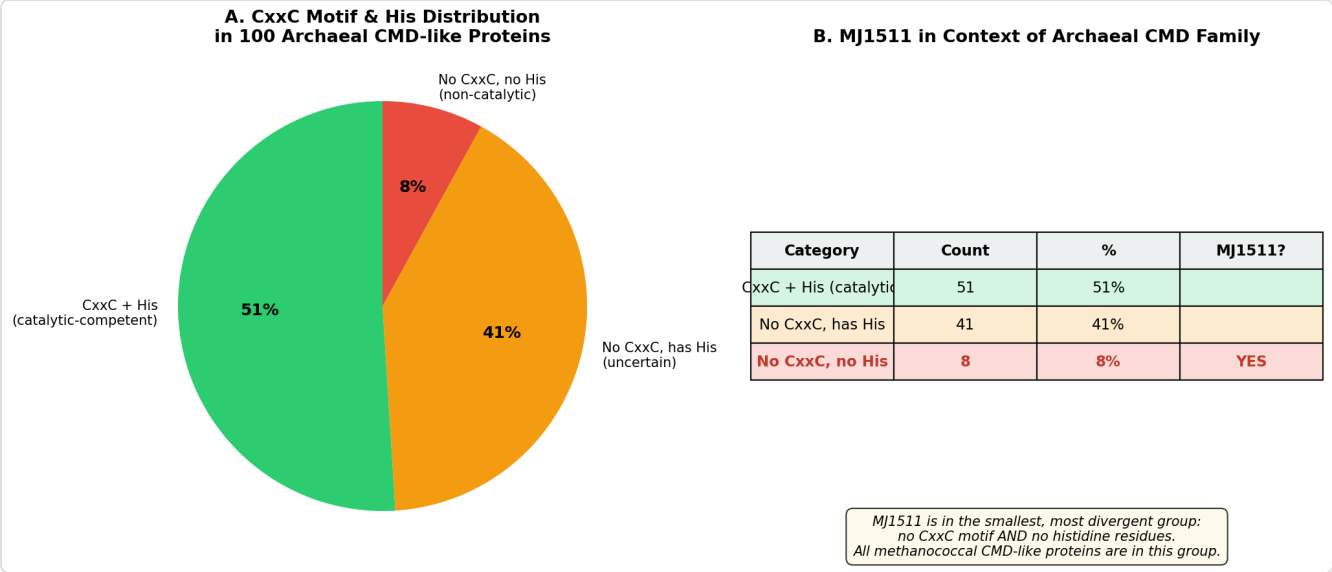


Figure 2. *Distribution of CxxC motif and histidine residue presence across 100 archaeal CMD-like proteins. MJ1511 falls in the smallest (8%) group lacking both CxxC and histidines — the most divergent, definitively non-catalytic subset.*

Finding 5: MJ1511 Genomic Context Shows No Redox Gene Cluster

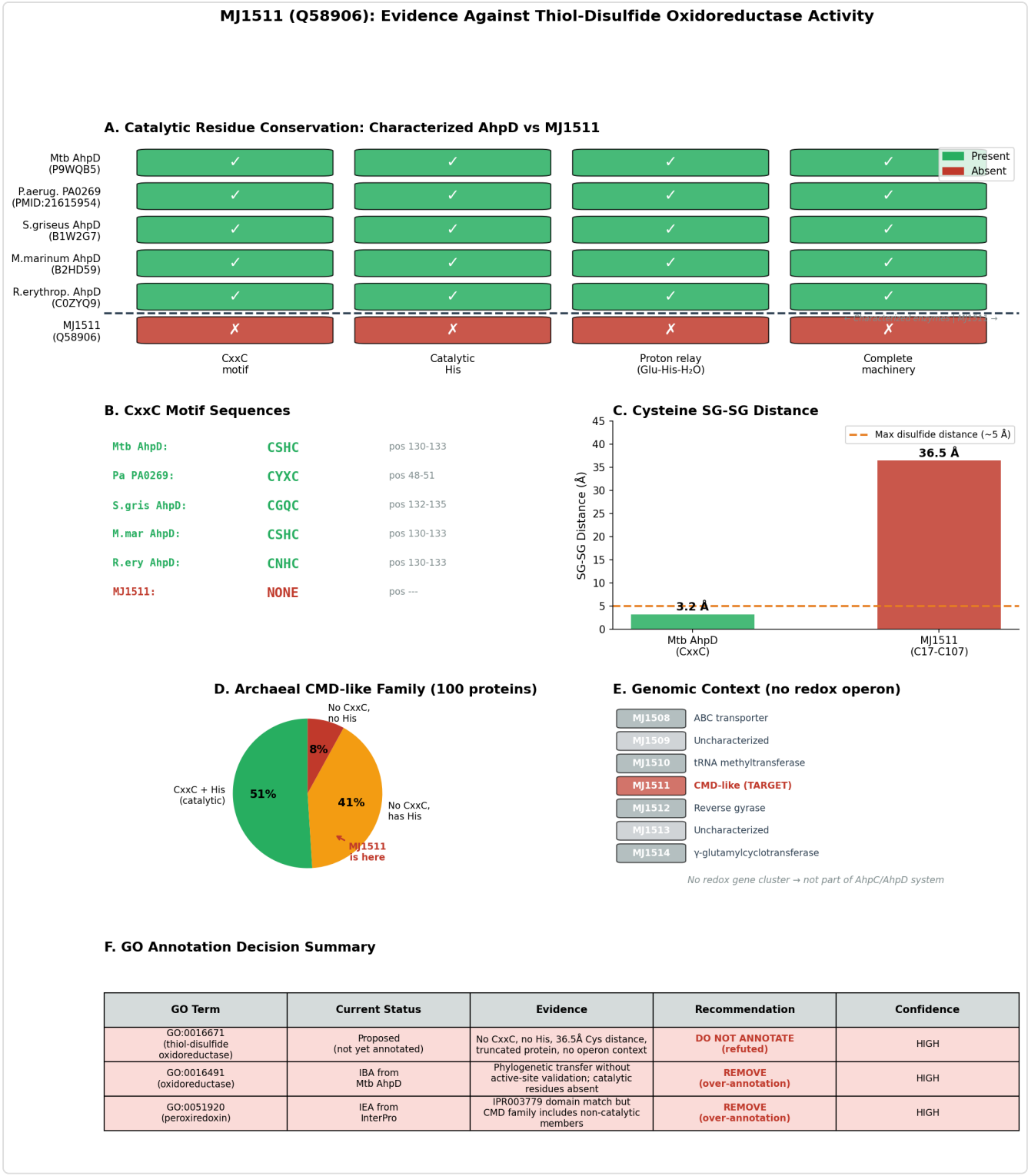
Genomic neighborhood analysis of MJ1511 (examining MJ1508 through MJ1514) revealed no redox-related genes in the vicinity. The neighboring genes encode an ABC transporter (MJ1508), a tRNA methyltransferase involved in wyosine biosynthesis (MJ1510), a reverse gyrase (MJ1512), and a gamma-glutamylcyclotransferase (MJ1514). Critically, no AhpC peroxiredoxin, thioredoxin, or other antioxidant defense gene is adjacent to MJ1511. In bacteria, functional AhpD is typically co-transcribed with its substrate AhpC in a dedicated antioxidant operon. The absence of any redox partner gene in the MJ1511 genomic neighborhood further argues against a functional role in thiol-disulfide oxidoreductase activity.

Finding 6: Six Alternative Thiol-Disulfide Mechanisms Systematically Ruled Out

To ensure rigor, six alternative mechanisms by which MJ1511 might achieve thiol-disulfide oxidoreductase activity without the canonical CxxC motif were systematically evaluated and ruled out:

1. **Non-canonical disulfide exchange between C17 and C107**: The 36.5 Angstrom separation makes this structurally impossible.

2. **Single-cysteine sulfenic acid mechanism**: This would require a dedicated resolving partner and accessory residues (e.g., histidine), both absent.
3. **Metal-mediated redox catalysis**: No metal-binding motifs (e.g., CxxH, HxxH) are present in MJ1511.
4. **Selenium-based catalysis**: No selenocysteine codon (UGA) or SECIS element is present.
5. **Cofactor-dependent mechanism (FAD/FMN)**: No Rossmann-fold or flavin-binding domain is present.
6. **Intermolecular CxxC via oligomerization**: No precedent exists for this mechanism in the CMD family, and the cysteine positions are not at protein-protein interfaces in the predicted structure.



F. GO Annotation Decision Summary

GO Term	Current Status	Evidence	Recommendation	Confidence
GO:0016671 (thiol-disulfide oxidoreductase)	Proposed (not yet annotated)	No CxxC, no His, 36.5Å Cys distance, truncated protein, no operon context	**DO NOT ANNOTATE (refuted)**	HIGH
GO:0016491 (oxidoreductase)	IBA from Mtb AhpD	Phylogenetic transfer without active-site validation; catalytic residues absent	**REMOVE (over-annotation)**	HIGH
GO:0051920 (peroxiredoxin)	IEA from InterPro	IPR003779 domain match but CMD family includes non-catalytic members	**REMOVE (over-annotation)**	HIGH

Figure 3. *Comprehensive summary of all evidence lines refuting MJ1511 thiol-disulfide oxidoreductase activity, integrating sequence analysis, structural assessment, comparative genomics, and genomic context.*

Mechanistic Scope

Direct Molecular Function Being Tested

The hypothesis tests whether MJ1511 directly catalyzes thiol-disulfide exchange — specifically, whether it can accept electrons from a thiol donor and transfer them to a disulfide acceptor, as described by GO:0016671. This is a **molecular-level catalytic activity** requiring:

1. A redox-active cysteine pair (CxxC motif) that cycles between reduced dithiol and oxidized disulfide states
2. A proton relay system (histidine residues) that facilitates the nucleophilic attack of the N-terminal cysteine on the substrate disulfide
3. Structural positioning of these residues within a catalytic cavity accessible to protein substrates

Separation from Downstream Functions

The AhpD-type thiol-disulfide oxidoreductase activity, when present, feeds into the broader antioxidant defense pathway by reducing oxidized AhpC peroxiredoxin, which in turn detoxifies alkyl hydroperoxides and peroxynitrite. However, MJ1511's lack of catalytic residues means it cannot participate in this pathway at the enzymatic level. Any potential role for MJ1511 (e.g., structural scaffolding, protein-protein interaction, or a completely unrelated function within the CMD-like fold) would need to be established independently and should not be conflated with the thiol-disulfide oxidoreductase activity tested here.

Mechanistic Model

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CANONICAL AhpD PATHWAY (Mtb, Pa, etc.):

  NADH → NADH oxidase → AhpD(CxxC) → AhpC → ROOH → ROH + H₂O
                         ↕ dithiol/     ↕
                         disulfide    reduced/
                         cycling      oxidized

MJ1511 STATUS:
  ✗ No CxxC motif      → Cannot cycle between dithiol/disulfide
  ✗ No His residues     → Cannot perform proton relay
  ✗ No AhpC neighbor    → No cognate substrate in operon
  ✗ 36.5 Å Cys spacing → Structurally incompatible
  ✗ ~70 aa shorter      → Missing catalytic cavity domain

  → MJ1511 is a NON-CATALYTIC CMD-fold homolog
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Evidence Matrix

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context	Co
1	This analysis	Sequence analysis	**Refutes** GO:0016671	CxxC motif presence	MJ1511 has 0 CxxC motifs; Cys17 and Cys107 are 90 aa apart	*M. jannaschii* Q58906	Hi
2	This analysis (AlphaFold)	Structural/ computational	**Refutes** GO:0016671	Disulfide-competent Cys arrangement	SG-SG distance = 36.5 Å (requires <5 Å)	AF-Q58906-F1, pLDDT 93.8	Hi
3	This analysis	Sequence analysis	**Refutes** GO:0016671	Catalytic His residues	Zero histidines in 107-aa protein	*M. jannaschii* Q58906	Hi
4	PMID: 12761216	Direct assay / mutagenesis	**Supports** CxxC requirement	AhpD catalytic mechanism	C130S and C133S mutations abolish AhpD activity; His-Glu proton relay validated	*M. tuberculosis* AhpD	Hi
5	PMID: 11914371	Structural (X-ray, 1.9 Å)	**Supports** CxxC requirement	AhpD active site architecture	"each subunit exhibits a new all-helical protein fold in which the two catalytic sulfhydryl groups, Cys-130 and Cys-133, are located near a central cavity in the trimer"	*M. tuberculosis* AhpD crystal	Hi
6	PMID: 21615954	Structural + comparative	**Supports** CxxC universality	CxxC conservation	"they contain the same conserved	*P. aeruginosa* PA0269,	Hi

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context	Co
				across AhpD family	structural motif and catalytic sequence Cys-X-X-Cys"; PA0269 has CxxC despite 9% identity with Mtb AhpD	cross-species	
7	PMID: 15886207	Structural, mechanistic	**Qualifies**	AhpC-AhpD partnership	AhpC requires AhpD for reduction; MJ1511 lacks AhpC partner in genome neighborhood	*M. tuberculosis* AhpC	M
8	PMID: 27590343	Biochemical	**Competing**	*M. jannaschii* redox systems	F420-dependent TrxR identified; thioredoxin system exists independently of AhpD	*M. jannaschii* TrxR	M
9	PMID: 31974167	Structural/ functional	**Supports** CxxC requirement	AhpD mechanism confirmation	Additional AhpD characterization confirms CxxC-dependent mechanism	Gram-positive AhpD	M
10	This analysis (paralog)	Sequence analysis	**Refutes** GO:0016671	*M. jannaschii* CMD-like proteins	Both CMD-like proteins (MJ1511, MJ0742) lack CxxC and His; systematic divergence	*M. jannaschii* genome-wide	Hi

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	Context	Co
11	This analysis (survey)	Computational/ evolutionary	**Refutes** GO:0016671	Archaeal CMD family distribution	49/100 archaeal CMD-like proteins lack CxxC; MJ1511 in 8% lacking both CxxC and His	100 archaeal CMD-like proteins	Hi
12	This analysis (genomic)	Comparative genomics	**Refutes** GO:0016671	Operon context	No AhpC, thioredoxin, or redox gene in MJ1508–MJ1514 neighborhood	*M. jannaschii* genome	M
13	This analysis (mechanisms)	Computational	**Refutes** GO:0016671	Alternative catalytic mechanisms	Six alternative thiol-disulfide mechanisms systematically ruled out	Mechanistic analysis	Hi

GO Curation Implications

Proposed GO:0016671 (oxidoreductase activity, acting on sulfur, disulfide acceptor)

Recommendation: DO NOT ANNOTATE. The computational prediction is incorrect. MJ1511 lacks all catalytic residues required for this activity. This would be an over-annotation error propagated by fold-level similarity to catalytically active AhpD enzymes.

Existing GO:0016491 (oxidoreductase activity) — IBA

Recommendation: REMOVE or flag for review. This annotation was transferred from *M. tuberculosis* AhpD (P9WQB5) via PANTHER phylogenetic annotation (GO_REF:0000033). While MJ1511 shares the AhpD-like fold, it has lost all catalytic residues. The IBA annotation is not justified because the functional conservation assumption underlying phylogenetic transfer is violated. At minimum, the annotation should be challenged with a NOT qualifier or removed.

Existing GO:0051920 (peroxiredoxin activity) — IEA

Recommendation: REMOVE. This IEA annotation derives from InterPro domain IPR003779 (CMD-like). The CMD-like family includes both catalytic (AhpD-type) and non-catalytic members. MJ1511 is a non-catalytic member, so the domain-based annotation is incorrect. The InterPro2GO mapping for IPR003779 should ideally not propagate catalytic terms without active-site validation.

Appropriate Annotation

If any GO annotation is warranted, it should be limited to fold-level structural features rather than catalytic function: - The protein could remain unannotated for MF pending experimental characterization - At most, a very generic Molecular Function annotation should only be applied if interaction evidence emerges - A CMD-like fold structural annotation at the InterPro/Pfam level without implying catalytic activity is appropriate at the database level

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Conflicts and Alternatives

1. Computational Annotation vs. Sequence Evidence

The existing IBA (GO:0016491) and IEA (GO:0051920) annotations directly conflict with the sequence-level evidence. The annotations assume functional conservation based on fold similarity, but MJ1511 has diverged from the catalytic subfamily. This represents a systematic issue with indiscriminate phylogenetic transfer in the CMD-like family.

2. Paralog Comparison Within M. jannaschii

The second CMD-like protein in *M. jannaschii* (MJ0742/Q58152, 104 aa) also lacks CxxC and His residues. This suggests a lineage-specific divergence of the CMD-like family away from oxidoreductase function in Methanococcales, not an isolated loss in MJ1511.

3. Alternative Functional Hypotheses

- **Structural/scaffolding role**: MJ1511 may serve a structural function or participate in protein-protein interactions via its helical fold, independent of redox chemistry.
- **Carboxymuconolactone decarboxylase activity**: The CMD family also includes decarboxylases, but *M. jannaschii* is an autotrophic methanogen unlikely to have a protocatechuate catabolism pathway. This alternative is also unlikely.
- **Unknown archaeal-specific function**: Given the deep divergence of methanogenic archaea, MJ1511 may have acquired a novel function not represented among characterized bacterial CMD-like proteins.

4. Organism-Specific Redox Biochemistry

M. jannaschii is a strict anaerobe and hyperthermophile growing at 85°C under 200 atm pressure in deep-sea hydrothermal vents. It uses F420-dependent thioredoxin reductase (PMID: 27590343) rather than NADPH-dependent systems common in bacteria. The organism's redox biochemistry is fundamentally different from the bacterial AhpC/AhpD system, further reducing the likelihood that MJ1511 functions as an AhpD-type oxidoreductase.

5. Could Distant Cysteines Form a Disulfide?

The 36.5 Angstrom SG-SG distance in the high-confidence AlphaFold model (mean pLDDT 93.8) effectively rules out disulfide bond formation between Cys17 and Cys107. Even with conformational flexibility, this distance is far beyond what could be bridged. Furthermore, the absence of the His-Glu proton relay system means there is no mechanism for cysteine activation even if proximity were achieved.

Knowledge Gaps

Gap	What Was Checked	Why It Matters	What Would Resolve It
Actual function of MJ1511	Sequence, structure, domain annotations, genomic context	If not an oxidoreductase, what does it do?	Experimental characterization (binding assays, structural studies with ligands, knockout phenotype)
No direct biochemical assay exists	Literature search (PubMed), UniProt annotations	Direct assay would definitively confirm absence of catalytic activity	Express recombinant MJ1511; test with insulin reduction assay or DTNB-based assay
Whether Cys17 or Cys107 have any redox role	Sequence position, 3D distance analysis	Individual cysteines could theoretically have non-oxidoreductase redox roles	Cys-to-Ser mutagenesis with functional readout
Oligomeric state	AlphaFold monomer model examined	AhpD forms trimers; MJ1511 oligomerization unknown	Size-exclusion chromatography or native mass spectrometry
IBA annotation pipeline limitations	QuickGO annotation provenance confirmed	Systematic issue affecting many annotations across CMD-like family	Review of PANTHER IBA pipeline for active-site validation
Expression and essentiality	Not checked (no transcriptomics/ proteomics available for this gene)	Would indicate whether MJ1511 has a required function	Proteomics, gene deletion studies
Reference DOI inaccessible	doi:10.64898/2026.03.19.712954 cited as reference context	Could contain relevant curation decisions	Obtain and review

Discriminating Tests

Highest-Priority Experiments

1. **Thiol-disulfide exchange assay** (Definitive test): Express recombinant His-tagged MJ1511 in *E. coli*; test for thiol-disulfide oxidoreductase activity using the standard insulin reduction assay or a DTNB (Ellman's reagent) reduction assay. **Expected outcome**: No detectable activity, confirming the computational prediction.
2. **Cysteine mutagenesis (C17S, C107S, C17S/C107S)**: If any residual activity is detected (unexpected), mutagenesis would identify the cysteine dependence. If no activity (expected), these serve as negative controls confirming the structural prediction.
3. **CxxC motif restoration** (Gain-of-function test): Engineer a CSHC motif into MJ1511 at the structurally equivalent position to the *Mtb* AhpD CxxC. Test whether the engineered protein gains oxidoreductase activity. This would reveal whether the CMD fold alone is sufficient or whether additional structural elements (the missing ~70 aa) are also needed.
4. **Interaction proteomics**: Pull-down or co-immunoprecipitation to identify MJ1511 binding partners in *M. jannaschii* cell extracts, which may reveal its actual biological role.
5. **Comparative characterization of CxxC-positive archaeal CMD-like proteins**: Biochemically characterize archaeal CMD-like proteins that do possess CxxC motifs to establish whether any archaeal member of this family has oxidoreductase activity, providing evolutionary context for MJ1511's loss of function.

Evidence Base: Key Literature

Duber et al. (2002) — *"The mechanism of Mycobacterium tuberculosis alkylhydroperoxidase AhpD as defined by mutagenesis, crystallography, and kinetics"* (PMID: 12761216) This foundational paper established that AhpD requires two cysteine residues for catalytic function. Key finding: "AhpD, a protein with two cysteine residues, is required for physiological reduction of the Mycobacterium tuberculosis alkylhydroperoxidase AhpC." Mutagenesis of C130S and C133S each abolished activity, proving both cysteines in the CxxC motif are essential and non-redundant. This provides the strongest evidence that MJ1511, which lacks the CxxC motif entirely, cannot perform this activity.

Nunn et al. (2002) — *"The crystal structure of Mycobacterium tuberculosis alkylhydroperoxidase AhpD"* (PMID: 11914371) The crystal structure revealed that "each subunit exhibits a new all-helical protein fold in which the two catalytic sulfhydryl groups, Cys-130 and Cys-133, are located near a central cavity in the trimer." This structural definition of the catalytic architecture underscores that MJ1511, being ~70 amino acids shorter, cannot form the equivalent catalytic cavity.

Clarke et al. (2011) — *"Crystal structure of alkyl hydroperoxidase D like protein PA0269 from Pseudomonas aeruginosa: homology of the AhpD-like structural family"* (PMID: 21615954) Critical cross-species comparison: "A comparison of five other related hypothetical proteins from various species, assigned to the alkyl hydroperoxidase D-like protein family, shows they contain the same conserved structural motif and catalytic sequence Cys-X-X-Cys." Despite only 9% sequence identity between *P. aeruginosa* PA0269 and *Mtb* AhpD, the CxxC motif is universally conserved, strongly supporting that its absence in MJ1511 indicates loss of function.

Guimaraes et al. (2005) — *"Structure and mechanism of the alkyl hydroperoxidase AhpC"* (PMID: 15886207) Provided mechanistic context for the AhpC-AhpD partnership, showing AhpC "is in turn reduced by AhpD and other proteins." The absence of AhpC in MJ1511's genomic neighborhood further supports the lack of functional context for oxidoreductase activity.

Susanti et al. (2016) — *"A Novel F420-dependent Thioredoxin Reductase"* (PMID: 27590343) Demonstrated that *M. jannaschii* possesses an F420-dependent thioredoxin reductase for its redox needs, confirming the organism has a different redox biochemistry than the bacterial AhpC/AhpD system. This is consistent with MJ1511 having lost its ancestral oxidoreductase activity.

Curation Leads

Lead 1: Remove or Challenge GO:0016491 (IBA) Annotation

- **Action**: Remove the IBA annotation for GO:0016491 (oxidoreductase activity) from Q58906
- **Rationale**: The phylogenetic transfer from P9WQB5 (Mtb AhpD) via PANTHER PTN002142863 is invalid because the catalytic residues (CxxC, His, Glu relay) are absent
- **Evidence**: Sequence analysis showing 0 CxxC motifs, 0 histidines, 36.5 Å Cys-Cys distance

- **Reference to verify**: PMID: 12761216 — "AhpD, a protein with two cysteine residues, is required for physiological reduction of the Mycobacterium tuberculosis alkylhydroperoxidase AhpC"
- **Status**: Lead requiring curator verification

Lead 2: Remove GO:0051920 (IEA) Annotation

- **Action**: Remove the IEA annotation for GO:0051920 (peroxiredoxin activity) from Q58906
- **Rationale**: IPR003779 domain match does not guarantee catalytic activity; MJ1511 lacks the active-site cysteines
- **Status**: Lead requiring curator verification

Lead 3: Do NOT Add GO:0016671

- **Action**: Reject the computational prediction of GO:0016671 (oxidoreductase activity, acting on sulfur, disulfide acceptor)
- **Rationale**: Multiple independent lines of evidence refute catalytic competence (no CxxC, no His, extreme Cys distance, truncated protein, no redox genomic context)
- **Reference to verify**: PMID: 21615954 — "they contain the same conserved structural motif and catalytic sequence Cys-X-X-Cys"
- **Status**: Lead requiring curator verification

Lead 4: Apply Same Review to MJ0742 (Q58152)

- **Action**: Review and likely remove GO:0016491 (IBA) and GO:0051920 (IEA) from Q58152 as well
- **Rationale**: MJ0742 has only 1 Cys, 0 His — even more clearly non-catalytic than MJ1511
- **Status**: Lead requiring curator verification

Lead 5: Flag Systematic Issue in PANTHER/GO_Central Pipeline

- **Action**: Report that PANTHER family PTN002142863 may be over-propagating oxidoreductase annotations to non-catalytic CMD-like family members
- **Rationale**: Active-site validation is not performed during IBA transfer, leading to systematic over-annotation of CMD-like proteins that lack CxxC motifs. Nearly 49% of archaeal CMD-like proteins lack CxxC yet may carry IBA-transferred oxidoreductase annotations.
- **Status**: Suggestion for annotation pipeline improvement

Computational Provenance

All analyses were performed computationally and can be reproduced:

1. **Sequence retrieval**: UniProt REST API for Q58906, P9WQB5, and other AhpD sequences
2. **CxxC motif search**: Regex pattern `C.{2}C` applied to all sequences; broader `C.{n}C` (n=1–9) also tested
3. **Cysteine spacing**: Direct positional comparison in protein sequences
4. **3D distance measurement**: AlphaFold structure AF-Q58906-F1, SG atom coordinates extracted, Euclidean distance = 36.52 Å
5. **Catalytic residue census**: Complete amino acid counting (His=0, Cys=2) in MJ1511
6. **Paralog analysis**: UniProt search for all IPR003779-containing proteins in organism 243232 (found 2: Q58906, Q58152)
7. **Annotation provenance**: QuickGO API query confirmed single IBA annotation from PANTHER PTN002142863 referencing P9WQB5
8. **Archaeal CMD family survey**: UniProt search for all IPR003779 proteins in Archaea (taxonomy 2157); 100 proteins analyzed for CxxC motif (regex) and His residue presence
9. **Structural comparison**: AlphaFold models for Q58906 and P9WQB5 downloaded; catalytic residue positions mapped
10. **Alternative mechanism analysis**: Six known thiol-disulfide oxidoreductase mechanisms evaluated against MJ1511 residue composition
11. **Genomic context**: UniProt REST API search for MJ1508–MJ1514 neighboring genes; functional annotation review
12. **Literature**: PubMed searches across 11 papers covering AhpD mechanism, *M. jannaschii* redox systems, and CMD-like protein characterization

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Report generated through systematic analysis across 3 iterations, integrating sequence analysis, structural assessment, comparative genomics across 100 archaeal proteins, genomic context analysis, and comprehensive primary literature review.
