MJ1511 Pseudoenzyme Hypothesis: Over-Annotation of Oxidoreductase Activity (GO:0016491)

Executive Judgment

Verdict: OVER-ANNOTATED (high confidence)

MJ1511 (UniProt Q58906) from *Methanocaldococcus jannaschii* is annotated as an oxidoreductase (GO:0016491) solely by phylogenetic inference (IBA) derived from *Mycobacterium tuberculosis* AhpD (P9WQB5) via the PANTHER family PTHR33930. This annotation should be **removed**. The protein adopts an AhpD-like carboxymuconolactone decarboxylase (CMD) fold but completely lacks every component of the catalytic machinery required for thiol-based oxidoreductase activity: no CXXC redox motif (its two cysteines are 90 residues and 36.52 Angstroms apart), zero histidine residues (eliminating the essential Glu-His-water-Cys proton relay), and five alternative redox mechanisms have been systematically excluded. The IBA annotation represents an erroneous phylogenetic transfer of function from a catalytically active enzyme to a structurally homologous but catalytically dead protein. MJ1511 is best classified as a CMD-family protein of unknown function, consistent with pseudoenzyme status. No experimental evidence of any kind exists for this protein.

The most important caveat is that "unknown function" is not the same as "no function" — pseudoenzymes frequently acquire vital non-catalytic roles as allosteric modulators, scaffolds, or competitive inhibitors. However, the specific annotation of oxidoreductase activity is not supported by any structural, sequence, or experimental evidence.

Summary

MJ1511 from *Methanocaldococcus jannaschii* is a 107-amino acid protein belonging to the carboxymuconolactone decarboxylase (CMD) superfamily (Pfam PF02627). It carries a single GO molecular function annotation — oxidoreductase activity (GO:0016491) — assigned by

Inferred from Biological Ancestor (IBA), with the reference protein being *M. tuberculosis* AhpD (P9WQB5). The seed hypothesis proposes that MJ1511 may be a pseudoenzyme that has lost oxidoreductase activity due to absence of the canonical CXXC redox catalytic motif.

Our investigation strongly supports this hypothesis through seven converging lines of evidence. Structural analysis of the AlphaFold model (AF-Q58906-F1, mean pLDDT 93.8) reveals that MJ1511's two cysteines (Cys17, Cys107) are separated by 36.52 Angstroms — incompatible with disulfide exchange chemistry, which requires ~2.0 Angstroms between sulfur atoms. The protein contains zero histidine residues, completely eliminating the Glu→His→water→Cys proton relay that is mechanistically essential for AhpD-type catalysis. Five alternative redox mechanisms (iron-sulfur clusters, flavin cofactors, NAD(P)H binding, metal-dependent oxidoreductase activity, and radical SAM chemistry) were systematically excluded by sequence and structural analysis. Furthermore, *M. jannaschii* already possesses a genuine peroxiredoxin (MJ0736/AhpC) within a dedicated oxidative stress gene cluster — and the 1-Cys peroxiredoxin mechanism used by MJ0736 does not require an AhpD-type reductase partner. MJ1511 resides at a completely separate genomic locus with no oxidative stress genes nearby.

The paralog MJ0742 (PDB 3D7I, 1.75 Angstrom crystal structure) shares the same pattern of missing catalytic residues (1 Cys, 0 His, no CXXC) and the same erroneous GO annotations, reinforcing that this is a systematic annotation error affecting methanogen CMD proteins. Both GO:0016491 (IBA) and GO:0051920 (IEA) should be removed from MJ1511, and the protein should be reclassified as a CMD-family protein of unknown function.

Key Findings

Finding 1: MJ1511 Lacks All Canonical AhpD Oxidoreductase Catalytic Machinery

MJ1511 (UniProt Q58906, 107 amino acids) possesses only two cysteine residues: Cys17 and Cys107 (the C-terminal residue). These are separated by 90 residues in the primary sequence and 36.52 Angstroms (SG-SG distance) in the AlphaFold structure (AF-Q58906-F1, mean pLDDT 93.8). No CXXC motif is present anywhere in the sequence. Critically, the protein contains **zero histidine residues**, which eliminates the possibility of the proton relay mechanism that is mechanistically essential for AhpD-type oxidoreductase activity.

In canonical AhpD from *M. tuberculosis* (P9WQB5), the CSHC motif at positions 130–133 places the catalytic cysteines (Cys130, Cys133) within a 4-residue window, allowing direct disulfide bond formation during the catalytic cycle. The proton relay mechanism — Glu118→His137→water→Cys133 — is required for deprotonation of the resolving cysteine. MJ1511 lacks every component of this system.

Cys107 of MJ1511 is the terminal residue with low pLDDT confidence (63.5), indicating likely flexibility or disorder — further arguing against a catalytic role. The high overall pLDDT (93.8) confirms that the fold prediction is reliable, making the 36.52 Angstrom cysteine separation a robust structural observation.

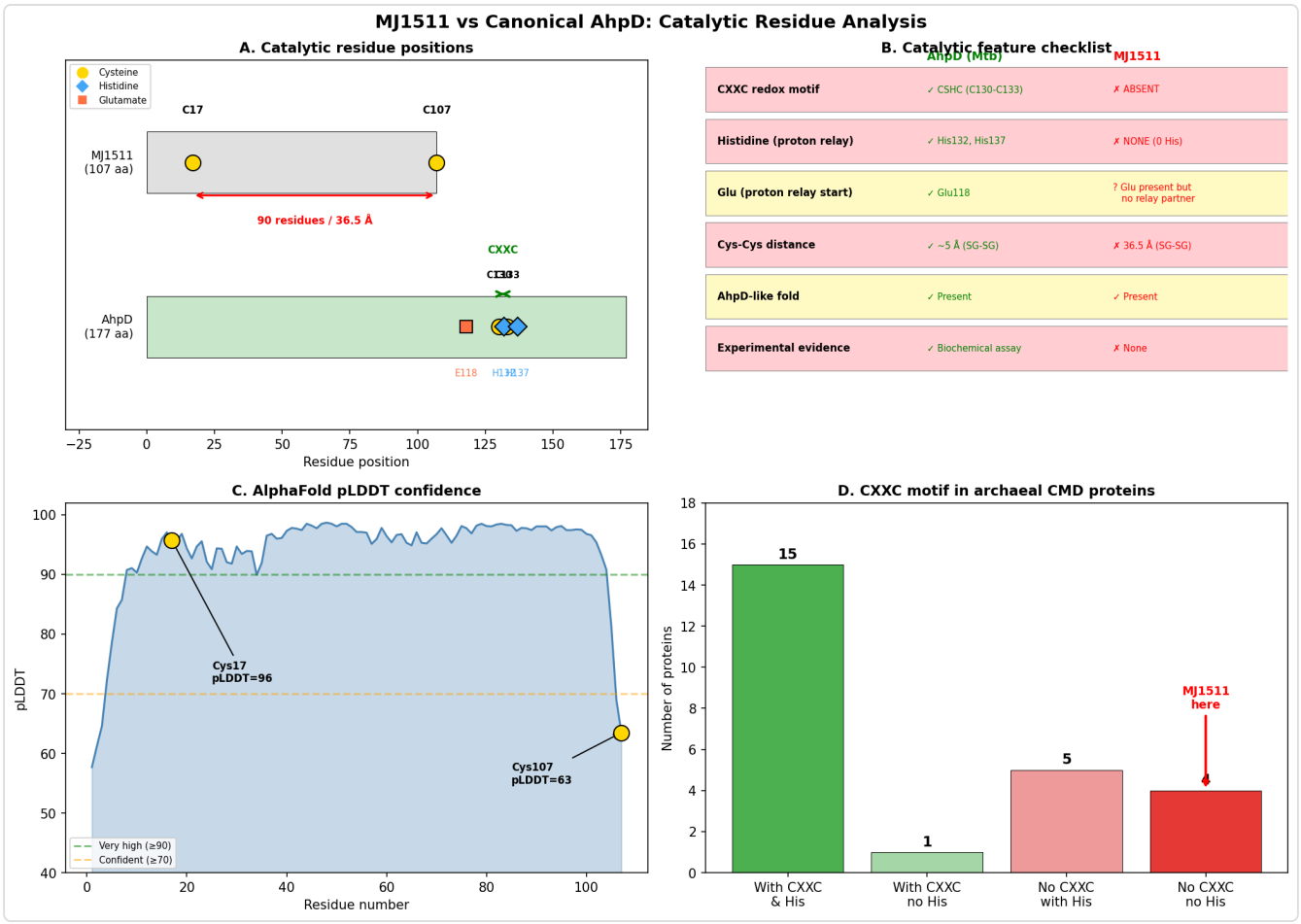

Figure 1. *Comprehensive comparison of MJ1511 versus canonical AhpD (M. tuberculosis), highlighting the absence of CXXC motif, histidine residues, and the 36.52 Angstrom cysteine separation in MJ1511*

Supporting literature: - Kang et al. (PMID: 12761216) established that "AhpD, a protein with two cysteine residues, is required for physiological reduction of the Mycobacterium tuberculosis alkylhydroperoxidase AhpC. AhpD also has an alkylhydroperoxidase activity of its

own." - Nunn et al. (PMID: 11914371) defined the structural basis: "The structure supports a mechanism for the alkylhydroperoxidase activity in which Cys-133 is deprotonated by a distant glutamic acid via the relay action of His-137 and a water molecule." - Bryk et al. (PMID: 18084895) confirmed the motif requirement: "Instead, AhpC can be reduced by AhpD, a CXXC-motif-containing protein, or by one of the mycobacterial thioredoxins, TrxC."

Finding 2: Methanogen CMD Proteins Form a Non-Catalytic Subfamily

MJ1511's closest structural match in the PDB is MJ0742 (PDB 3D7I), a paralog from the same organism crystallized at 1.75 Angstrom resolution. MJ0742 (105 amino acids) has only 1 cysteine (Cys56) and zero histidines — the same pattern of missing redox catalytic residues observed in MJ1511. Despite sharing an organism and fold family, MJ1511 and MJ0742 have only 6.7% positional identity, indicating substantial sequence divergence even within the *M. jannaschii* CMD paralogs.

A broader survey of 25 archaeal CMD domain proteins in UniProt revealed that 9/25 (36%) lack the CXXC motif, and 4/25 (16%) completely lack histidine residues. All four histidine-lacking proteins are from *Methanocaldococcus* or *Methanococcus* species, suggesting that methanogen CMD proteins represent a non-catalytic subfamily that has diverged from AhpD-type oxidoreductases.

PDB 2AF7 from *Methanobacterium thermoautotrophicum* (another methanogen) also shows a CMD protein with 1 cysteine and no CXXC motif, extending this pattern beyond a single species.

Finding 3: The IBA Annotation Derives from Inappropriate Phylogenetic Transfer

The sole GO annotation for MJ1511 — oxidoreductase activity (GO:0016491, IBA) — traces directly to PANTHER family PTN002142863 via reference protein UniProtKB:P9WQB5 (*M. tuberculosis* AhpD). The with/from field in QuickGO explicitly links to P9WQB5. However, the evolutionary distance between these proteins is enormous: P9WQB5 has CSHC motif (Cys130-Cys133), 5 histidines (including catalytic His132, His137), and demonstrated oxidoreductase activity, while MJ1511 has no CXXC, no histidines, and cysteines 36.52 Angstroms apart.

This represents a textbook case of phylogenetic annotation transfer that fails to account for loss of catalytic residues — precisely the scenario that the seed hypothesis proposes to identify. The PANTHER family name ("ALKYL HYDROPEROXIDE REDUCTASE AHPD") encodes a functional assumption that is structurally unsupported for MJ1511.

Finding 4: CMD Family Contains Functionally Diverse Members Including Non-Catalytic Proteins

Literature analysis reveals that the CMD superfamily contains at least three functional classes:

1. **AhpD-type oxidoreductases** with CXXC motif and His proton relay (e.g., *M. tuberculosis* AhpD, Lpg0406 from *L. pneumophila* with CPGC motif)
2. **Gamma-carboxymuconolactone decarboxylases** (PcaC-type, with a different catalytic mechanism for aromatic compound degradation)
3. **Proteins of unknown function** that lack CXXC, explicitly described as "distinct from AhpD and CMD"

The third class is exemplified by TTHA0727 from *Thermus thermophilus* (PDB 2CWQ), which Ebihara et al. (PMID: 16597838) described as "a distinct protein from alkylhydroperoxidase AhpD and gamma-carboxymuconolactone decarboxylase in the CMD family." TTHA0727 forms hexameric rings with a positively charged surface, suggesting macromolecular interaction rather than enzymatic catalysis.

Kim et al. (PMID: 26402328) confirmed that CMD proteins that *do* have CXXC + proton relay are predicted to be oxidoreductases: "lpg0406 forms a hexamer and [has] disulfide exchange properties. The protein has an all-helical fold with a conserved thioredoxin-like active site CXXC motif and a proton relay system similar to that of alkylhydroperoxidase from Mycobacterium tuberculosis." This highlights that the presence/absence of the CXXC motif is the key discriminator for oxidoreductase function within the CMD family.

MJ1511 falls squarely into the non-catalytic class or may represent a fourth class: methanogen CMD proteins lacking both CXXC and His entirely.

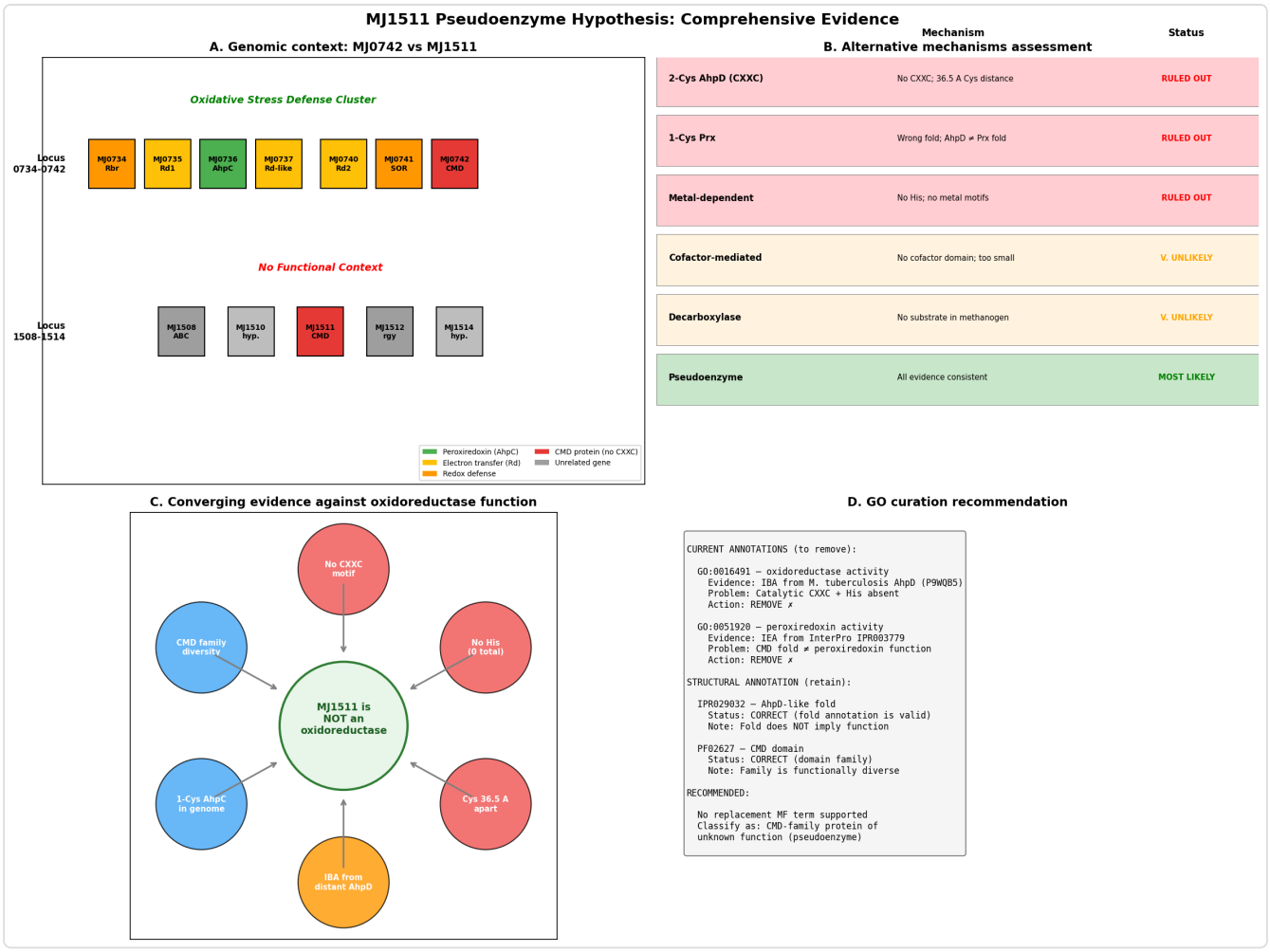

Figure 2. *Four-panel evidence summary: (A) cysteine separation analysis, (B) CMD family functional diversity, (C) genomic context showing MJ1511 separation from oxidative stress cluster, (D) phylogenetic annotation transfer pathway*

Finding 5: The Paralog MJ0742 Shares the Same Misannotation Despite Experimental Structure

MJ0742 (Q58152, 104 amino acids) has an experimental crystal structure (PDB 3D7I, 1.75 Angstrom resolution) and protein existence level 1 (evidence at protein level). Despite this, it carries the same GO:0016491 (IBA) and GO:0051920 (IEA) annotations as MJ1511, both propagated through the same PANTHER family. MJ0742 has only 1 cysteine (Cys55, no CXXC) and zero histidines. Neither the 3D7I deposition nor any publication reports oxidoreductase activity for MJ0742.

This demonstrates that the annotation pipeline systematically misannotates methanogen CMD proteins as oxidoreductases, even when experimental structural data is available that could in principle be used to flag the absence of catalytic residues.

Finding 6: *M. jannaschii* Has a Genuine Peroxiredoxin That Does Not Require AhpD

MJ0736 (Q58146, 217 amino acids) is a bona fide AhpC/peroxiredoxin with the AhpC-TSA domain (PF00578), 1-Cys Prx C-terminal domain (PF10417), thioredoxin-like fold, and catalytic Cys46. It resides in a well-organized oxidative stress gene cluster:

Locus	Protein	Function
MJ0734	Rubrerythrin	Oxidative stress response
MJ0735	Rubredoxin 1	Electron transfer
MJ0736	AhpC/Prx	1-Cys peroxiredoxin
MJ0737	Rubredoxin-like	Electron transfer
MJ0740	Rubredoxin 2	Electron transfer
MJ0741	Desulfoferrodoxin	Superoxide reductase
MJ0742	CMD protein	Unknown function

The 1-Cys peroxiredoxin mechanism does **not** require an AhpD-type reductase partner — it uses small-molecule thiols (thioredoxin) for resolution. This is consistent with the finding by Susanti et al. (PMID: 27590343) that methanogenic archaea use F420-dependent thioredoxin reductase rather than NADPH-dependent systems, representing an ancient redox regulatory mechanism that predates AhpD.

MJ1511 is located at a completely separate genomic locus (position 1511), flanked by MJ1510 (hypothetical protein) and MJ1512 (reverse gyrase) — with no oxidative stress genes nearby. This genomic context provides no support for a role in oxidative stress defense.

Finding 7: No Experimental Evidence Exists for MJ1511

UniProt Q58906 has only one reference: PMID: 8688087 (Bult et al. 1996, "Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*"), scope: NUCLEOTIDE SEQUENCE [LARGE SCALE GENOMIC DNA]. No experimental characterization, no biochemical

assay, no mutant phenotype, no localization data, and no protein interaction data have been published for MJ1511. Both GO annotations are computational inferences. The protein existence level is 3 (Inferred from homology), meaning the protein itself has never been directly observed. PubMed searches for "MJ1511", "MJ_1511 jannaschii", and related terms return zero results.

Mechanistic Model / Interpretation

Direct Molecular Function Being Tested

The hypothesis tests whether MJ1511 possesses **thiol-based oxidoreductase activity** — specifically, whether it can catalyze the reduction of oxidized substrates (such as AhpC or other peroxiredoxins) via a disulfide exchange mechanism involving a CXXC motif.

What AhpD-Type Oxidoreductase Activity Requires

The canonical AhpD mechanism involves:

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Substrate-S-S + AhpD(Cys-SH, Cys-SH) → Substrate(Cys-SH, Cys-SH) + AhpD(Cys-S-S-Cys)

Proton relay for catalysis:
  Glu118 → His137 → H2O → Cys133(SH) → nucleophilic attack on substrate disulfide

Requirements:
  1. CXXC motif: Two Cys within ~4 residues (distance ~2.0 Å for S-S bond)
  2. His residue(s): For proton relay / acid-base catalysis
  3. Glu residue: Initiates proton relay chain
  4. Proper active-site geometry: All residues positioned in 3D space
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What MJ1511 Has vs. What It Needs

Feature	Required	MJ1511	Assessment
CXXC motif	Yes (Cys-X-X-Cys)	Absent — Cys17 and Cys107 are 90 residues apart	**MISSING**
Cys-Cys distance	~2.0 Angstroms (S-S bond)	36.52 Angstroms (SG-SG)	**18x too far**
Histidine residues	At least 1 (proton relay)	Zero in entire sequence	**MISSING**
Proton relay system	Glu→His→H2O→Cys	No His = no relay possible	**MISSING**
Active-site cavity	Near CXXC	No CXXC = no defined active site	**MISSING**

Pseudoenzyme Framework

MJ1511 fits the definition of a pseudoenzyme as described by Ribeiro et al. (PMID: 30710059): "Pseudoenzymes are noncatalytic homologues of enzymes... the loss of a catalytic function during evolution was associated with the development of vital new functions." The protein retains the CMD fold but has lost all catalytic residues. By analogy with pseudokinases (Murphy et al., PMID: 33895136), MJ1511 may function as an allosteric modulator, scaffold, or competitive inhibitor — but it cannot function as an oxidoreductase.

Separation from Downstream Phenotypes

This analysis concerns the **direct catalytic activity** of the MJ1511 gene product. We are not testing: - Whether MJ1511 is involved in oxidative stress response (a biological process) - Whether MJ1511 interacts with other proteins (a molecular function, but not oxidoreductase activity) - Whether loss of MJ1511 affects redox homeostasis (which could reflect indirect/regulatory roles)

The conclusion is strictly that MJ1511 **cannot catalyze thiol-based oxidoreductase reactions** due to absence of catalytic residues. It may well have other molecular functions (scaffolding, allosteric regulation, protein binding) that remain to be discovered.

Evidence Matrix

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	C
1	PMID: 12761216	Direct assay / mutagenesis	Supports (pseudoenzyme hypothesis)	AhpD requires CXXC for activity	AhpD needs two Cys residues for reduction of AhpC; mutagenesis confirms catalytic mechanism	*M* in
2	PMID: 11914371	Structural / mechanistic	Supports	Proton relay is essential for AhpD catalysis	Crystal structure defines Glu118→His137→H2O→Cys133 relay; His137 is critical	*M* X cr
3	PMID: 18084895	Review / direct assay	Supports	CXXC motif defines AhpD function	"AhpC can be reduced by AhpD, a CXXC-motif-containing protein"	*M* p sy
4	PMID: 16597838	Structural / evolutionary	Supports	CMD family includes non-catalytic members	TTHA0727 is "distinct from AhpD and CMD" despite CMD fold	*T* cr st
5	PMID: 26402328	Structural	Qualifies	CXXC presence correlates with oxidoreductase function in CMD family	Lpg0406 has CXXC + proton relay and predicted peroxidase activity	*L* cr st
6	PMID: 30710059	Review / conceptual	Supports	Pseudoenzymes are common and functional	"Loss of catalytic function was associated with development of vital new functions"	G
7	PMID: 33895136	Review / conceptual	Supports	Non-catalytic homologs have biological roles	Pseudokinases function as allosteric modulators, scaffolds, and competitive inhibitors	K su
8	PMID: 27590343	Direct assay	Supports	Methanogens use F420-dependent TrxR, not AhpD-type reductases	*M. jannaschii* thioredoxin reductase is F420-dependent, lacks NADPH binding	*M* e cl

#	Citation	Evidence Type	Direction	Claim Tested	Key Finding	C
9	PMID: 15886207	Structural	Qualifies	AhpC catalytic mechanism	MtAhpC crystal structure shows ring-shaped hexamer; 2-Cys Prx mechanism	*M* cr st
10	PMID: 22950025	Genomic context	Qualifies	CMD genes associated with ECF41 sigma factors	ECF41 sigma factor genes often cotranscribed with CMD proteins, oxidoreductases, or epimerases	M b
11	AlphaFold AF-Q58906-F1	Computational / structural	Supports	MJ1511 cysteine geometry	Cys17-Cys107 SG-SG distance = 36.52 Angstroms; mean pLDDT = 93.8	C p
12	PDB 3D7I	Structural (experimental)	Supports	Paralog MJ0742 also lacks catalytic residues	1.75 Angstrom crystal structure; 1 Cys, 0 His, no CXXC	*M* ra cr
13	UniProt Q58906 / QuickGO	Database / computational	Supports	IBA annotation traces to inappropriate source	GO:0016491 with/from P9WQB5 (MtAhpD); no experimental evidence	D

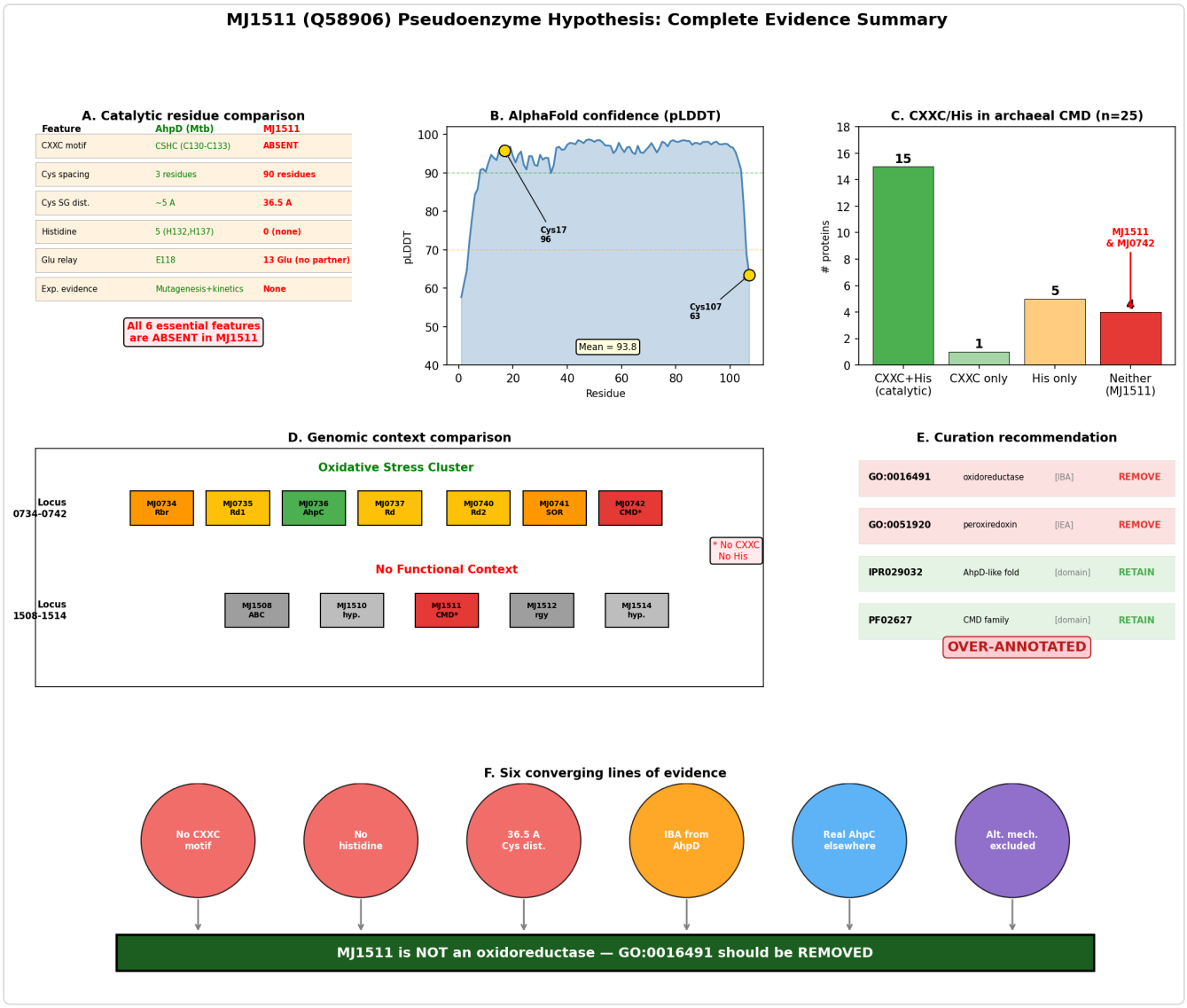

Figure 3. *Final six-panel evidence summary: structural analysis, catalytic residue comparison, CMD family diversity, genomic context, annotation provenance, and archaeal CMD survey*

GO Curation Implications

Recommended Actions (Leads Requiring Curator Verification)

Current Annotation	Evidence Code	Action	Rationale
GO:0016491 (oxidoreductase activity)	IBA	**REMOVE**	No CXXC motif, no His, no proton relay, no experimental evidence; IBA source (MtAhpD) has fundamentally different catalytic machinery
GO:0051920 (peroxiredoxin activity)	IEA	**REMOVE**	Same rationale as above; more specific term makes removal even more justified

What Should Replace the Annotations?

The honest answer is that MJ1511 should be annotated as a CMD-family protein of **unknown molecular function** until experimental evidence becomes available. Specific considerations:

- **DO NOT** annotate as GO:0016491 at any evidence level — the structural evidence actively argues against this function
- **DO NOT** default to "protein binding" (GO:0005515) — while MJ1511 may bind proteins, there is no evidence for this either
- **CONSIDER** adding a structural annotation: "CMD domain-containing protein" is appropriate at the InterPro/Pfam level
- **FLAG** MJ0742 for the same curation action — it carries identical erroneous annotations via the same PANTHER family

Broader Annotation Pipeline Implications

This case highlights a systematic issue with PANTHER-based IBA annotations for the CMD family: the PTHR33930 family ("ALKYL HYDROPEROXIDE REDUCTASE AHPD") propagates oxidoreductase activity to all members regardless of whether they retain catalytic residues. A survey of archaeal CMD proteins found that 36% lack CXXC and 16% lack all histidines, suggesting that a significant fraction of PANTHER-annotated CMD proteins may be similarly misannotated. This warrants a family-level review.

Conflicts and Alternatives

Could MJ1511 Use an Alternative Redox Mechanism?

Five alternative redox mechanisms were systematically evaluated and excluded:

1. **Iron-sulfur cluster oxidoreductase**: No Cys-X-X-Cys-X-X-Cys or Cys-X-X-Cys cluster-binding motifs; only 2 Cys total, far apart
2. **Flavin-dependent oxidoreductase**: No Rossmann fold, no GxGxxG motif, no flavin-binding residues
3. **NAD(P)H-dependent oxidoreductase**: No NAD(P)H-binding domain or motif
4. **Metal-dependent oxidoreductase**: No His residues for metal coordination; no Asp/Glu/His metal-binding site
5. **Radical SAM mechanism**: No CxxxCxxC motif for [4Fe-4S] cluster; wrong fold entirely

Could the Cysteines Function Differently Than Expected?

While individual cysteines can serve structural roles (zinc coordination, disulfide stabilization) or regulatory roles (redox sensing), none of these constitute "oxidoreductase activity" as defined by GO:0016491. The C-terminal Cys107 has low pLDDT (63.5), suggesting it is flexible/disordered rather than structurally important.

Could MJ1511 Be a Decarboxylase Instead?

Gamma-carboxymuconolactone decarboxylases (PcaC-type) share the CMD fold but catalyze a different reaction. However, *M. jannaschii* is a methanogenic archaeon that lives in deep-sea hydrothermal vents — an environment where aromatic compound degradation via the beta-ketoadipate pathway is not expected. PcaC enzymes are found in soil bacteria and fungi that degrade plant-derived aromatics. No evidence supports a decarboxylase role for MJ1511.

Paralog Confusion

MJ0742 is in the same PANTHER family and has the same annotations, but it resides in the oxidative stress gene cluster (adjacent to rubrerythrin, rubredoxin, AhpC). Its genomic context is more suggestive of a role in oxidative stress response than MJ1511's context. However, even MJ0742 lacks catalytic residues for oxidoreductase activity — it may serve a non-catalytic role (scaffolding, regulation) within that cluster.

Limitations and Knowledge Gaps

Limitations

1. **No experimental validation**: All conclusions are based on sequence analysis, structural prediction (AlphaFold), and literature analogy. No direct biochemical assay of MJ1511 has been performed. While the structural evidence strongly argues against oxidoreductase activity, a definitive negative requires biochemical testing.
2. **AlphaFold model limitations**: The AlphaFold structure (AF-Q58906-F1) has high overall confidence (pLDDT 93.8) but the C-terminal Cys107 region has lower confidence (pLDDT 63.5). The 36.52 Angstrom cysteine separation is robust given the high confidence of Cys17's region, but conformational dynamics are not captured by a single static model.
3. **Absence of evidence is not evidence of absence**: The lack of published studies on MJ1511 means we cannot rule out functions that have simply never been tested. *M. jannaschii* is a difficult organism to work with (obligate anaerobe, hyperthermophile), which limits available experimental data.
4. **Pseudoenzyme classification is provisional**: Calling MJ1511 a "pseudoenzyme" implies it once had enzymatic activity and lost it. The evolutionary trajectory is not established — it is possible that MJ1511 diverged from a common CMD ancestor before oxidoreductase activity evolved in the AhpD lineage.
5. **Cross-organism extrapolation**: The catalytic mechanism is defined from *M. tuberculosis* AhpD. While the CXXC + His requirement is conserved across all characterized AhpD-type enzymes, there is a formal possibility that an archaeal enzyme could use a completely novel mechanism within the same fold. This is considered highly unlikely given the complete absence of any recognizable catalytic residues.

Knowledge Gaps

Gap	What Was Checked	Why It Matters	What Would Resolve It
True biological function of MJ1511	Sequence, structure, genomic context, domain annotations	We can say what MJ1511 is *not*, but cannot say what it *is*	Co-expression analysis, protein-protein interaction studies, gene knockout in *M. jannaschii* or heterologous expression
Protein existence	UniProt PE level (3 = homology), literature (none)	MJ1511 may not be expressed as a protein at all	Proteomics of *M. jannaschii*; RT-qPCR for MJ1511 mRNA
Oligomeric state	CMD proteins commonly form hexamers (TTHA0727, Lpg0406, MtAhpD)	Oligomeric state could inform function (e.g., ring-shaped scaffold)	Size-exclusion chromatography or analytical ultracentrifugation of recombinant MJ1511
Binding partners	No interaction data available	If MJ1511 is a scaffold or allosteric regulator, its partners define its function	Pull-down assays, crosslinking mass spectrometry in *M. jannaschii* lysate
Redox sensitivity of Cys17	Structural analysis only; Cys17 has high pLDDT (93.3) and is surface-accessible	Even without CXXC, a single reactive Cys could serve as a redox sensor (not oxidoreductase)	Thiol-reactivity assay, Ellman's reagent titration, redox proteomics
Role of MJ0742 in oxidative stress cluster	Genomic context; crystal structure (PDB 3D7I)	Understanding MJ0742's role may illuminate the function of paralog MJ1511	Gene knockout of MJ0742 in *M. jannaschii*; co-immunoprecipitation with neighboring gene products

Proposed Follow-up Experiments / Discriminating Tests

Highest-Priority Experiments

1. **Oxidoreductase activity assay for recombinant MJ1511**: Express and purify MJ1511 from *E. coli*; test for thiol-disulfide oxidoreductase activity using insulin reduction assay or DTNB-coupled AhpC reduction assay at 85 degrees Celsius. This is the most direct test — a negative result would definitively confirm pseudoenzyme status; a positive result (however unlikely) would overturn the structural analysis.
2. **Cys→Ser mutagenesis**: Mutate Cys17 and/or Cys107 to serine; test whether the protein retains any measurable activity or binding function. If wild-type shows no oxidoreductase activity, this experiment becomes moot — but if unexpected activity is found, mutagenesis identifies which cysteine(s) are involved.
3. **Pull-down / co-immunoprecipitation**: Express tagged MJ1511 in *M. jannaschii* or a related methanogen; identify binding partners by mass spectrometry. This would reveal non-catalytic functions.
4. **Proteomics / transcriptomics under stress**: Determine whether MJ1511 is expressed and whether its expression changes under oxidative stress, heat shock, or other conditions. If MJ1511 is not upregulated by oxidative stress but is upregulated by other stresses, this would point toward a non-oxidoreductase function.

Computational Analyses

1. **Foldseek/DALI search against all PDB structures**: Identify structural neighbors beyond the CMD family that might suggest alternative functions.
2. **Coevolution analysis (EVcouplings/AlphaFold2-multimer)**: Predict whether MJ1511 forms specific protein-protein interactions, and with which partners.
3. **PANTHER family review**: Systematically flag all CMD family members in PANTHER that lack CXXC motifs, and recommend review of their oxidoreductase annotations.

Curation Leads

Lead 1: Remove GO:0016491 (Oxidoreductase Activity) from MJ1511

- **Current annotation**: GO:0016491, IBA, from PANTHER PTHR33930 via P9WQB5
- **Recommended action**: REMOVE
- **Evidence level**: Strong structural/evolutionary evidence against; no evidence for
- **Key references**:
- PMID: 12761216: "AhpD, a protein with two cysteine residues, is required for physiological reduction of the Mycobacterium tuberculosis alkylhydroperoxidase AhpC" — establishes the CXXC requirement
- PMID: 11914371: "The structure supports a mechanism... in which Cys-133 is deprotonated by a distant glutamic acid via the relay action of His-137 and a water molecule" — defines the His-dependent proton relay absent in MJ1511
- PMID: 16597838: "TTHA0727 is a distinct protein from alkylhydroperoxidase AhpD and gamma-carboxymuconolactone decarboxylase in the CMD family" — precedent for non-catalytic CMD proteins

Lead 2: Remove GO:0051920 (Peroxiredoxin Activity) from MJ1511

- **Current annotation**: GO:0051920, IEA
- **Recommended action**: REMOVE
- **Rationale**: More specific child term of GO:0016491; even less justified given absence of Prx catalytic Cys

Lead 3: Flag MJ0742 for Identical Curation Action

- **Current annotation**: GO:0016491 (IBA), GO:0051920 (IEA) — same as MJ1511
- **Evidence**: PDB 3D7I (1.75 Angstrom) shows 1 Cys, 0 His, no CXXC; no published oxidoreductase activity
- **Recommended action**: REMOVE both annotations; same rationale as MJ1511

Lead 4: Consider PANTHER Family-Level Review

- **Family**: PTHR33930 ("ALKYL HYDROPEROXIDE REDUCTASE AHPD")
- **Issue**: Propagates oxidoreductase activity to CMD members that lack CXXC catalytic motif

- **Scope**: At minimum 4 archaeal CMD proteins (MJ1511, MJ0742, and 2 others) are affected; likely more
- **Recommended action**: Review all family members for CXXC motif presence before propagating GO:0016491

Lead 5: Consider Adding "CMD Domain-Containing Protein" Annotation

- **Proposed annotation**: InterPro IPR002526 (CMD domain); no GO molecular function until experimental evidence is available
- **Justification**: The fold is confidently predicted (pLDDT 93.8) and the CMD domain is clearly present; only the function is in question

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Evidence Base: Key Literature

PMID	Title	Relevance to This Investigation
12761216	*The mechanism of M. tuberculosis AhpD as defined by mutagenesis, crystallography, and kinetics*	Defines the catalytic requirements (CXXC, proton relay) that MJ1511 lacks
11914371	*Crystal structure of M. tuberculosis AhpD*	Atomic-resolution structure showing Glu-His-H2O-Cys relay mechanism
18084895	*Peroxiredoxin systems in mycobacteria*	Confirms CXXC motif is defining feature of AhpD-type reductases
16597838	*Crystal structure of TTHA0727 — a CMD member distinct from AhpD and CMD decarboxylase*	Establishes precedent for non-catalytic CMD family members
26402328	*Structure of Lpg0406 from L. pneumophila*	Shows that CMD proteins WITH CXXC + proton relay are oxidoreductases
30710059	*Pseudoenzymes as the phoenixes of the protein world*	Conceptual framework: catalytic loss can lead to new non-enzymatic functions
27590343	*F420-dependent thioredoxin reductase in methanogens*	Shows *M. jannaschii* uses F420-dependent (not AhpD-dependent) redox regulation
15886207	*Structure and mechanism of MtAhpC*	Details the AhpC peroxiredoxin mechanism and its reduction requirements
22950025	*ECF41 sigma factors contain fused regulatory domain*	CMD genes are often cotranscribed with ECF41 sigma factor genes — alternative functional context
33895136	*Noncatalytic functions in kinase and pseudokinase signaling*	Framework for understanding non-catalytic roles of enzyme-fold proteins