AIGR Gene Hypothesis Deep Research — Final Report

Target: *pmp20* (O14313), *Schizosaccharomyces pombe* — Peroxiredoxin Activity Prediction

Focus type: computational_prediction **Hypothesis slug:** prediction-peroxiredoxin-activity **Terms under evaluation:** peroxiredoxin activity (GO:0051920), peroxidase activity (GO:0004601), hydrogen peroxide catabolic process (GO:0042744)

Executive Judgment

Verdict: PARTIALLY SUPPORTED — the pseudoenzyme/"lost resolving cysteine" alternative is REFUTED, but direct catalytic activity in *S. pombe* remains unproven.

The BioReason-Pro SFT prediction that pmp20 is a peroxiredoxin/peroxidase is grounded in real, intact catalytic machinery — not a degenerate over-annotation. The seed hypothesis raised a specific, testable structural concern: that pmp20 might have lost the resolving cysteine (Cr) or otherwise degenerated its active site, which would make the peroxiredoxin predictions a pseudoenzyme artifact. That specific concern is **incorrect on its premise**. pmp20 is a **1-Cys peroxiredoxin** — a legitimate, well-characterized structural subclass of the peroxiredoxin family (PMP20/PRDX6 type) that normally has no resolving cysteine at all. The absence of a second cysteine is therefore not degeneration; it is the expected, catalytically competent architecture. Structural analysis confirms that the peroxidatic active site is fully intact and correctly assembled.

However, the prediction cannot be scored as fully "supported" because the single direct enzymatic assay performed on *S. pombe* PMP20 failed to detect thioredoxin-dependent peroxidase activity, and the authors instead proposed a molecular chaperone role (PMID: 20356456). Family-level evidence (orthologs in *Hansenula polymorpha*, human, and mouse) strongly supports antioxidant/peroxiredoxin function, but 1-Cys peroxiredoxins typically use physiological reductants other than thioredoxin (e.g., glutathione, ascorbate, or protein

partners), so a negative thioredoxin assay does not by itself rule out peroxidase activity. The net result for a curator: the molecular machinery justifies retaining GO:0051920 and GO:0042744 as **conservation/family-supported leads**, but the species-specific claim of *thioredoxin* peroxidase activity (GO:0008379) is not supported and should be generalized or dropped for this organism.

The most important caveat is the tension between **structural competence** (strongly in favor of peroxidase function) and **the one direct functional assay** (which was negative for the thioredoxin-coupled reaction). This is a classic 1-Cys peroxiredoxin situation where the correct reductant/partner matters, and it is exactly the kind of nuance a curator should preserve rather than flatten into a single yes/no annotation.

Key Findings

Finding 1 — pmp20 retains an intact peroxidatic cysteine motif but is a 1-Cys peroxiredoxin (no resolving Cys)

UniProt O14313 encodes a 156-amino-acid protein containing **exactly one cysteine, Cys43**, which UniProt annotates as the active-site residue forming a "Cysteine sulfenic acid (–SOH) intermediate." This is the diagnostic signature of the peroxidatic cysteine (Cp) of a peroxiredoxin: during catalysis, Cp attacks the peroxide substrate and is oxidized to a sulfenic acid intermediate.

Critically, Cys43 sits within an **intact peroxiredoxin peroxidatic motif**: the local sequence P36-G-A-F-T40-P-P-C43 matches the canonical **P-x-x-x-(T/S)-x-x-C** signature exactly. Position 1 is proline (P36), position 5 is the conserved threonine (T40), and position 8 is the peroxidatic cysteine (C43). All three invariant anchor residues of the motif are satisfied.

Because the entire 156-residue sequence contains **no second cysteine anywhere**, there is **no resolving cysteine (Cr)**. This is the defining feature of the **1-Cys peroxiredoxin** subclass. In 2-Cys peroxiredoxins, Cr forms a disulfide with the oxidized Cp to complete the catalytic cycle; in 1-Cys peroxiredoxins, that role is filled instead by an external low-molecular-weight thiol or a protein partner. The seed hypothesis framed the absence of a resolving cysteine as possible "degeneration" indicative of a pseudoenzyme — but for the PMP20/PRDX6 family, the lack of Cr is the **normal, catalytically legitimate architecture**, not a loss.

Domain and family classifications corroborate the assignment: InterPro places pmp20 in the **PRX5-like** superfamily (IPR037944) with a thioredoxin-like fold, and PANTHER assigns it to **PTHR10430 PEROXIREDOXIN:SF39 (PMP20)**. Both are canonical peroxiredoxin family memberships, not degenerate outliers.

Finding 2 — The PMP20 family is a genuine antioxidant peroxiredoxin, but *S. pombe* pmp20 showed no thioredoxin-dependent peroxidase activity in vitro

Family-level functional evidence is consistent and strong:

- In the methylotrophic yeast *Hansenula polymorpha*, deletion of the peroxisomal peroxiredoxin (pmp20Δ) produces a methanol-dependent growth defect, elevated reactive oxygen species, lipid peroxidation, loss of peroxisome membrane integrity, and necrotic cell death (PMID: 18694816). This is a direct loss-of-function demonstration that a PMP20-family peroxiredoxin protects against oxidative damage.
- Human and murine PMP20 orthologs "exhibit antioxidant activity in vitro" and share high sequence similarity to thiol-specific antioxidant proteins (PMID: 10514471).

However, the **species-specific** evidence for *S. pombe* introduces an important qualification. In a direct comparative study of fission yeast peroxiredoxin isozymes and glutathione peroxidase, recombinant *S. pombe* PMP20 showed **no thioredoxin-dependent peroxidase activity**, in contrast to the TPx, BCP, and GPx enzymes that were assayed in parallel. The authors explicitly stated that "peroxidase activity was not observed for PMP20 (peroxisomal membrane protein 20)" and proposed that "the fission yeast PMP20 without thioredoxin-dependent peroxidase activity may act as a molecular chaperone" (PMID: 20356456).

Two interpretations are compatible with this negative result, and a curator should hold both:

1. **Wrong reductant.** 1-Cys peroxiredoxins characteristically do *not* use thioredoxin as their recycling partner; they typically depend on glutathione, ascorbate, or specific protein partners. A negative thioredoxin-coupled assay is therefore weak evidence against peroxidase activity per se — it may simply reflect the wrong electron donor in the in vitro reconstitution.
2. **Genuinely divergent function.** Alternatively, *S. pombe* pmp20 may have shifted toward a chaperone-dominant role, as the authors suggest, even while retaining the structural active site.

The evidence does not currently discriminate cleanly between these, which is precisely why the prediction lands at "partially supported."

Finding 3 — AlphaFold model shows a fully assembled peroxiredoxin catalytic tetrad (Pro36–Thr40–Cys43–Arg122)

The high-confidence AlphaFold model **AF-O14313-F1-v6** (overall mean pLDDT 95.4; active-site residues pLDDT 93.9–97.9) directly refutes the "degenerate active site" premise of the seed hypothesis. Measured geometry of the modeled active site shows the conserved catalytic residues correctly positioned around the peroxidatic cysteine:

Interaction	Measured distance	Interpretation
Cys43(SG) – Thr40(OG1)	3.00 Å	Hydrogen-bonding distance; Thr correctly poised to stabilize the Cp thiolate/transition state
Cys43(SG) – Arg122(guanidinium CZ)	3.86 Å	Conserved catalytic Arg correctly positioned to lower Cp pKa and stabilize the sulfenate
Pro36	Present, high confidence	Completes the canonical Pro–Thr–Cys–Arg tetrad

The **Pro36–Thr40–Cys43–Arg122 catalytic tetrad** — the universally conserved active-site constellation of functional peroxiredoxins — is therefore fully assembled at high modeling confidence. There is no second cysteine anywhere in the structure, confirming the 1-Cys architecture at the structural level and matching the sequence analysis. Structurally, pmp20 is an **active-site-competent peroxiredoxin**, not a degenerate pseudoenzyme.

Mechanistic Model / Interpretation

The three findings converge on a coherent picture. pmp20 is a **structurally intact 1-Cys peroxiredoxin** whose catalytic machinery is fully present, but whose recycling chemistry in *S. pombe* is unresolved.

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      PEROXIREDOXIN CATALYTIC CYCLE (1-Cys type)

ROOH (H2O2 / lipid peroxide)
     |
     v
Cp-SH  --(peroxidatic Cys43 attacks peroxide)-->  Cp-SOH  + ROH
(thiolate                                          (sulfenic acid
 stabilized by                                      intermediate;
 Thr40 + Arg122)                                    UniProt-annotated)
     ^                                                  |
     |                                                  v
     |                                Resolution step requires an EXTERNAL reductant
     |                                (1-Cys Prx has NO resolving Cys of its own)
     |                                                  |
     +---- reduced by: glutathione / ascorbate / -------+
           protein partner  (NOT thioredoxin in vitro — <a href="https://pubmed.ncbi.nlm.nih.

Catalytic tetrad (AlphaFold AF-O14313-F1-v6, pLDDT ~95):
    Pro36 --- Thr40 (3.0 Å to Cp) --- Cys43(Cp) --- Arg122 (3.9 Å to Cp)
    ALL PRESENT AND CORRECTLY POSITIONED
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What the machinery tells us (strongly supported): The peroxidatic half-reaction — the step that actually defines "peroxidase/peroxiredoxin activity" — has an intact, correctly geometried active site. Sequence (P-x-x-x-T-x-x-C motif), family classification (PRX5-like, PANTHER PMP20), and structure (assembled Pro/Thr/Cys/Arg tetrad) all agree. The seed's pseudoenzyme hypothesis is refuted.

What remains uncertain (the resolution half-reaction): The absence of a resolving cysteine is normal for this subclass, but it means the enzyme depends on an external reductant. The only direct *S. pombe* assay used thioredoxin and was negative — an expected outcome if pmp20 is a glutathione- or ascorbate-dependent 1-Cys enzyme, but also consistent with a functional shift toward chaperone activity. The negative thioredoxin result should therefore **not** be read as "no peroxidase activity," only as "no *thioredoxin-dependent* peroxidase activity."

The correct mechanistic scope for curation is: pmp20's **immediate molecular function** is thiol-based peroxide reduction via a sulfenic-acid intermediate on Cys43. Downstream/pleiotropic consequences at the family level (peroxisome membrane protection, prevention of lipid peroxidation and necrotic death in orthologs) are real but should not be conflated with the direct MF term.

Evidence Matrix

Citation	Evidence type	Supports / Refutes / Qualifies	Claim tested	Key finding	Context	Cor & li
UniProt O14313 (database record)	Structural/ evolutionary; database	Refutes pseudoenzyme; supports Prx MF	Does pmp20 retain the Cp motif and a resolving Cys?	Single Cys (C43) in intact P36-x-x-x-T40-x-x-C43 motif; annotated Cys-sulfenic-acid intermediate; 1-Cys architecture (no Cr)	*S. pombe*, sequence-level	Hig seq fact ann infe bas
InterPro IPR037944 / PANTHER PTHR10430:SF39 (database)	Computational/ family	Supports Prx family membership	Is pmp20 a bona fide peroxiredoxin family member?	PRX5-like, thioredoxin-like fold; PANTHER PMP20 subfamily	*S. pombe*	Hig clas doe pro acti
AlphaFold AF-O14313-F1-v6 (structural model)	Structural (predicted)	Refutes degenerate active site	Is the catalytic tetrad assembled?	Pro36–Thr40–Cys43–Arg122 tetrad intact; Cp–Thr 3.0 Å, Cp–Arg 3.9 Å; pLDDT ~95	*S. pombe*, in silico	Hig con pre exp stru
PMID: 20356456	Direct in vitro assay	Qualifies / partially refutes	Does *S. pombe* PMP20 have Trx-dependent peroxidase activity?	No thioredoxin-dependent peroxidase activity observed; proposed	*S. pombe* recombinant protein	Hig neg res red a st con 1-C

Citation	Evidence type	Supports / Refutes / Qualifies	Claim tested	Key finding	Context	Co & li
				chaperone role		
PMID: 18694816	Mutant phenotype (loss-of-function)	Supports (family level)	Does a PMP20-family Prx protect against oxidative damage?	pmp20Δ → ROS, lipid peroxidation, membrane leakage, necrotic death	*Hansenula polymorpha*	Hig ort *S. p* phe dow of M
PMID: 10514471	Direct assay (ortholog)	Supports (family level)	Do PMP20 orthologs have antioxidant activity?	Human/ murine PMP20 show antioxidant activity in vitro; similar to thiol-specific antioxidants	Human, mouse	Mo hig ort spe tra infe

GO Curation Implications

Lead requiring curator verification. The molecular and structural evidence justifies treating the peroxiredoxin function as real but not fully experimentally nailed down in *S. pombe*.

GO term	Aspect	Recommended action (lead)	Rationale
GO:0051920 peroxiredoxin activity	MF	**Retain as conservation/ family-supported lead** (evidence code ISS/IBA-type reasoning)	Intact Cp motif + assembled catalytic tetrad + family membership strongly support the MF; direct *S. pombe* kinetics not demonstrated
GO:0004601 peroxidase activity	MF	**Retain as the broader parent lead**	Supported by same structural/ family evidence; more general and safer than the thioredoxin-specific child
GO:0042744 hydrogen peroxide catabolic process	BP	**Retain as family-supported lead**	Consistent with peroxiredoxin MF and ortholog oxidative-stress phenotypes
GO:0008379 thioredoxin peroxidase activity	MF	**Do NOT assign / generalize** for *S. pombe*	Directly contradicted by the one in vitro assay (PMID: 20356456); 1-Cys Prx generally are not thioredoxin-coupled
Chaperone activity (e.g., unfolded protein binding / chaperone)	MF	**Flag as a competing/ complementary lead**	Proposed by PMID: 20356456; needs curator judgement on whether experimentally established

The evidence supports an **MF** (peroxiredoxin/peroxidase) and an associated **BP** (H2O2 catabolism), with the peroxisomal **CC** context suggested by family membership (PMP20 = peroxisomal membrane protein 20). "Protein binding" is not an appropriate final term here; the informative MF is peroxiredoxin/peroxidase activity. The key curator decision is whether to attach these as computationally/conservation-supported (ISS/IBA) rather than experimentally supported (IDA), given the absence of a positive direct assay in this species.

Mechanistic Scope

- **Direct gene-product activity (what is being tested):** Thiol-based reduction of peroxide substrate via nucleophilic attack by the peroxidatic Cys43, forming a Cys-sulfenic-acid intermediate stabilized by Thr40 and Arg122. This is the immediate molecular function.
- **Resolution chemistry:** As a 1-Cys peroxiredoxin, pmp20 lacks an internal resolving cysteine and must be recycled by an external reductant (glutathione/ascorbate/protein partner), not by an intramolecular disulfide.
- **Downstream / not to be conflated with MF:** Peroxisome membrane integrity, protection from lipid peroxidation, prevention of necrotic death (ortholog phenotypes) — these are pathway-level consequences of the antioxidant function, valuable as BP context but not direct MF evidence.
- **Competing direct function:** Possible molecular chaperone activity, proposed on the basis of the absent thioredoxin-dependent peroxidase activity in *S. pombe*.

Conflicts and Alternatives

1. **Structural competence vs. negative functional assay.** The dominant conflict: sequence + AlphaFold say "fully competent active site," while the one direct *S. pombe* assay says "no thioredoxin-dependent peroxidase activity." The most parsimonious reconciliation is that the assay used the wrong reductant for a 1-Cys enzyme, not that the enzyme is inactive.
2. **Chaperone reassignment.** PMID: 20356456 proposes a molecular chaperone role. This is a genuine alternative primary function that a curator should weigh; peroxiredoxins are known to moonlight as chaperones, so the two are not mutually exclusive.
3. **Ortholog/paralog transfer risk.** The strongest positive functional data come from *H. polymorpha*, human, and mouse orthologs, not *S. pombe* itself. Cross-species function transfer is reasonable for a conserved family but is inference, not direct evidence.
4. **"Lost resolving cysteine = pseudoenzyme" is a category error.** The seed hypothesis implicitly treats a missing Cr as pathological. For the PMP20/PRDX6 1-Cys subclass this is the normal architecture, so the pseudoenzyme framing does not apply.

Limitations and Knowledge Gaps

Gap	What was checked	Why it matters	What would resolve it
No positive direct peroxidase assay in *S. pombe*	Literature; found only a negative thioredoxin-coupled assay	Determines whether MF should be IDA vs ISS/IBA	Peroxide-reduction assay with glutathione/ascorbate or candidate protein reductants
Physiological reductant unknown	Sequence shows 1-Cys architecture (needs external reductant)	1-Cys Prx recycling partner defines its true activity	Reconstitution with GSH/glutaredoxin, ascorbate, and protein-partner screens
Chaperone vs peroxidase primacy unresolved	PMID: 20356456 proposes chaperone	Affects which MF is "core"	Side-by-side chaperone and peroxidase assays; in vivo H2O2 sensitivity of pmp20Δ
Subcellular localization not independently verified here	Family name implies peroxisomal (PMP20)	CC term assignment	GFP localization / peroxisome fractionation in *S. pombe*
AlphaFold is a model, not experimental structure	pLDDT ~95 gives high confidence	Geometry-based active-site claims rest on a prediction	Experimental crystal/cryo-EM structure

Discriminating Tests

1. **Reductant-swap peroxidase assay.** Repeat the peroxide-reduction assay using **glutathione (± glutaredoxin) and ascorbate** as reductants rather than thioredoxin. A positive result would convert the annotation from a conservation lead to a direct (IDA) peroxiredoxin annotation and explain the negative thioredoxin result.
2. **Cys43-to-Ser active-site mutant.** Test whether C43S abolishes any detected peroxidase (and/or chaperone) activity — establishes that the annotated Cp is the catalytic residue.

3. **In vivo oxidative-stress phenotyping.** Compare *S. pombe* wild-type vs pmp20Δ (and C43S knock-in) under H2O2 and organic-peroxide challenge; scored ROS, lipid peroxidation, and viability — mirrors the informative *H. polymorpha* experiment.
4. **Chaperone activity assay.** Aggregation-prevention (e.g., citrate synthase/insulin turbidity) to test the chaperone hypothesis directly and determine whether it is redox-state dependent.
5. **Localization confirmation.** Fluorescent tagging / peroxisome co-localization to justify a peroxisomal CC term.

Curation Leads

All items below are leads requiring curator verification.

Candidate references with exact snippets to verify: - PMID: 20356456 — verify snippet: *"peroxidase activity was not observed for PMP20 (peroxisomal membrane protein 20)"* and *"The fission yeast PMP20 without thioredoxin-dependent peroxidase activity may act as a molecular chaperone."* → supports NOT assigning GO:0008379 and flags a competing chaperone MF. - PMID: 18694816 — verify snippet: *"absence of the peroxisomal peroxiredoxin leads to loss of peroxisome membrane integrity and necrotic cell death"* → family-level antioxidant/peroxidase support (ortholog). - PMID: 10514471 — verify PMP20 orthologs *"exhibit antioxidant activity in vitro"* → family-level support.

Candidate GO actions: - Retain **GO:0051920 peroxiredoxin activity** and **GO:0004601 peroxidase activity** as conservation/family-supported (ISS/IBA) leads, not IDA. - Retain **GO:0042744 hydrogen peroxide catabolic process** as a supporting BP lead. - Do **not** assign **GO:0008379 thioredoxin peroxidase activity** for *S. pombe*; generalize to the parent peroxidase term. - Consider a peroxisomal **CC** term consistent with PMP20 family identity (verify by localization). - Consider flagging a **chaperone** MF as an alternative/complementary function pending direct evidence.

Suggested curator questions: - Is there any positive peroxidase assay for *S. pombe* pmp20 with a non-thioredoxin reductant? - Should the record carry both a peroxiredoxin MF (conservation-based) and a chaperone MF (experiment-based proposal)?

Suggested experiments: the five discriminating tests above, prioritizing the glutathione/ascorbate-coupled peroxidase assay and the C43S mutant.

Bottom Line

pmp20 is a structurally intact **1-Cys peroxiredoxin** with a complete, correctly assembled Pro36–Thr40–Cys43–Arg122 catalytic tetrad. The seed's "lost resolving cysteine / pseudoenzyme over-annotation" hypothesis is **refuted** — the missing resolving cysteine is the normal architecture of this subclass, not degeneration. The BioReason-Pro peroxiredoxin/peroxidase/H2O2-catabolism predictions are therefore **partially supported**: justified as conservation/family-based leads (retain GO:0051920, GO:0004601, GO:0042744), but not upgraded to direct-experimental confidence because the only direct *S. pombe* assay found no thioredoxin-dependent peroxidase activity (GO:0008379 should be generalized/dropped for this organism), and a competing chaperone role remains on the table.