DnaK (P0A6Y8) Zinc-Ion-Binding Prediction — Focused Curation Report

Gene: DnaK (Hsp70 chaperone) · *Escherichia coli* K-12 · UniProt **P0A6Y8** **Prediction under review:** BioReason-Pro SFT → **zinc ion binding (GO:0008270)** **Focus type:** computational_prediction · **Slug:** prediction-zinc-binding **Reference context:** doi:10.64898/2026.03.19.712954

Executive Judgment

Verdict: Over-annotated / weakly supported — treat as a non-core, likely artifactual assignment (effectively refuted as a molecular function).

DnaK does not possess a bona fide zinc-coordinating center. The prediction is not wholly baseless — there is a single existing IDA GO annotation (GO:0008270) on DnaK — but that annotation rests entirely on **one low-specificity, abundance-biased proteomic Zn-blot screen** (P 11985624) and is contradicted by DnaK's sequence and structure:

- DnaK has **only one cysteine (Cys15)** in 638 residues and **no CXXC / C4 zinc-finger motif** — it is structurally incapable of forming a classical cysteine-based zinc site.
- **0 of 63 DnaK PDB entries** contain a bound zinc ion, despite extensive structural characterization of both the NBD and SBD.
- The **genuine zinc-binding component of the DnaK/DnaJ/GrpE system is the co-chaperone DnaJ** (P08622), which has a CR-type zinc finger coordinating 2 Zn^{2+} via 8 cysteines. The prediction is best explained as **paralog/system confusion plus database carry-over**.

Recommended lead: **do not curate GO:0008270 as a DnaK function**; if retained at all, downgrade to a low-confidence, in-vitro-only/adventitious note, not a mechanistic MF.

Most important caveat: an existing curated IDA annotation does exist (EcoliWiki,), P 11985624 so this is a case of challenging a weak legacy annotation rather than a term with zero support. The judgment rests on the low specificity of that assay and the absence of any structural/sequence basis.

Evidence Matrix

Citation	Evidence type	Stance	Claim tested	Key finding	Context
UniProt P0A6Y8 (computed here)	Sequence/ computational	**Refutes**	DnaK has a Cys-based zinc motif	Only 1 Cys (Cys15); 0 CXXC motifs; no Metal-binding/Zinc-finger feature	*E. coli* DnaK, 6 aa
RCSB (63 entries, queried here)	Structural	**Refutes**	Zinc is present in DnaK structures	0/63 DnaK PDB entries contain a ZN ligand	NBD + S crystal/ NMR structur
P 11985624 (Katayama 2002)	Direct assay (proteomic ^{65}Zn-blot)	**Qualifies (sole support)**	DnaK binds Zn^{2+} in vitro	DnaK "newly identified" among 9 hits alongside AckA, GlyA, TktA/B, Tsf, ribosomal proteins	2D-gel Z blot of total *E.* lysate
QuickGO annotation record (queried here)	Database	**Qualifies**	Provenance of DnaK Zn annotation	Only GO:0008270 on DnaK = IDA/ EcoliWiki/ P 11985624; identical ref also used for DnaJ	Annotat metada
P 15683252 (Shi 2005)	Structural/ biochemical	**Competing (DnaJ)**	Which system member binds Zn	DnaJ has two C4-type zinc fingers (C144/147/161/164/183/186/197/200) binding 2 Zn^{2+}	*E. coli* D
P 8662861 (Banecki 1996)	Direct assay (AAS/HgS titration)	**Competing (DnaJ)**	DnaJ zinc stoichiometry	Two Zn^{2+} per DnaJ monomer	*E. coli* D
P 23708608 (Qi 2013);	Structural	**Refutes (by omission)**	DnaK core MF is Zn-dependent	Full-length ATP-bound and domain structures: ATPase NBD + peptide SBD; no zinc site	*E. coli* DnaK

Citation	Evidence type	Stance	Claim tested	Key finding	Context
34453889; 33950017					
InterPro (queried Iter 2)	Structural/ evolutionary	**Refutes**	DnaK has a zinc-finger domain	Only Hsp70 domains (IPR012725, PF00012, NBD/SBD/PBD); no zinc-finger/metal domain	*E. coli* DnaK
Ortholog Cys census (queried Iter 2)	Evolutionary	**Refutes**	Zinc binding conserved in Hsp70	DnaK orthologs carry 0–5 Cys, 0 CXXC; *M. tuberculosis* DnaK has **0 cysteines**	Hsp70 family (bacteri yeast/ human)

GO Curation Implications

- **GO:0008270 (zinc ion binding, MF):** Lead — **do not propagate / recommend removal or NOT-qualifier consideration** for DnaK. The single IDA source is a low-specificity proteomic screen; there is no motif, no structural zinc, and no mechanistic role. If curator policy forbids removing an IDA, at minimum it should **not** be treated as a core molecular function and should carry an explicit caveat that it derives from a whole-proteome Zn-blot with probable non-specific binding.
- **Do not** substitute a vaguer term (e.g., "metal ion binding" or "protein binding") — no informative metal-binding term is supported.
- **Well-supported core MF terms to prefer/retain instead:** ATP binding (GO:0005524), ATP hydrolysis activity (GO:0016887), ADP binding (GO:0043531), unfolded protein binding (GO:0051082), ATP-dependent protein folding chaperone (GO:0140662). These reflect DnaK's actual activities.
- **Paralog note for curators:** the zinc-ion-binding annotation is biologically correct **for DnaJ (P08622)**, and the shared

P 11985624

reference suggests the DnaK entry is a co-annotation carry-over.

Mechanistic Scope

The immediate molecular function being tested is **direct coordination of a Zn^{2+} ion by DnaK**. DnaK's actual direct activities are: (i) ATP binding and hydrolysis in the N-terminal nucleotide-binding domain (actin/hexokinase fold; uses Mg^{2+} and K^{+}, not Zn^{2+}), and (ii) binding of exposed hydrophobic segments of unfolded clients in the C-terminal substrate-binding domain, allosterically coupled to the nucleotide state. No step of this cycle requires or involves a structural zinc. Any zinc signal observed in a lysate blot is a **downstream in vitro observation** on an abundant protein, not a mechanistic feature of the chaperone cycle.

Conflicts and Alternatives

- **Paralog confusion (most likely):** DnaJ, the obligate co-chaperone in the DnaK/DnaJ/GrpE system, genuinely binds 2 Zn^{2+} via a CR-type zinc finger. Zinc binding is a real property of the *system* but resides in DnaJ.
- **Database carry-over / frequency bias:** the same reference (P 11985624) underlies the IDA on both DnaK and DnaJ; a model trained on such annotations would plausibly transfer "zinc ion binding" onto the more famous/abundant DnaK.
- **Assay artifact:** ^{65}Zn-blotting of 2D gels captures many abundant, acidic, or surface-exposed proteins non-specifically; the paper's own hit list (kinases, transketolase, ribosomal proteins, EF-Ts) is enriched for proteins with no canonical zinc-finger, signaling low specificity.
- **No organism/isoform escape hatch:** DnaK is a single-copy, well-conserved bacterial Hsp70; no isoform harbors extra cysteines that could form a zinc site.
- **Negative literature check (Iter 3):** targeted PubMed searches for a *functional* DnaK zinc/metal-binding site or a copper/zinc redox role returned no primary evidence (the only zinc-finger chaperone hit was thioredoxin-2, unrelated to DnaK). No study proposes zinc as

mechanistically required for DnaK. The UniProt BP term "stress response to copper ion" (GO:1990169) reflects a physiological stress response, not direct metal coordination by DnaK.

Knowledge Gaps

1. **Is the Katayama Zn signal specific or adventitious?** Checked: sequence (1 Cys), structure (0/63 PDB), and hit-list composition all argue non-specific. Matters because it is the sole primary support. Resolve with: purified DnaK ICP-MS/atomic-absorption zinc stoichiometry and competition/specificity controls (as were done for DnaJ).
2. **Does any DnaK conformer transiently bind Zn functionally?** Checked: allosteric-cycle structures show no zinc site. Matters only if a regulatory zinc were proposed. Resolve with: metal-content analysis across ADP/ATP states.
3. **What exactly does EcoliWiki's IDA claim?** Checked provenance (P 11985624 only). Matters for whether removal vs. caveat is appropriate. Resolve with: read full Katayama methods to confirm DnaK's measured affinity/specificity class.

Discriminating Tests

- **ICP-MS / atomic absorption on purified recombinant DnaK** (apo vs. Zn-loaded) — quantify Zn:protein stoichiometry; expect ~0 for a genuine non-binder, ~2 for DnaJ control.
- **Cys15→Ser mutant + metal blot** — a genuine Cys-based site would lose signal; a non-specific surface interaction would not.
- **Side-by-side ^{65}Zn-blot of DnaK vs DnaJ vs a bona fide C4 zinc-finger control** with EDTA/competitor titration to grade specificity.
- **AlphaFold3/metal-aware docking or MIB/CHED metal-site prediction** on P0A6Y8 — expect no high-confidence zinc site (bioinformatic corroboration).

Curation Leads (require curator verification)

- **Action change:** Flag GO:0008270 on DnaK as **not a core function**; propose removal or downgrade with an explicit provenance caveat. Do **not** replace with "metal ion binding" or "protein binding."
- **Candidate references to verify (exact snippets):**
- P 11985624
  — *"nine zinc-binding proteins were newly identified including: acetate kinase (AckA), DnaK, serine hydroxymethyltransferase (GlyA)..."* (verify this is the sole basis and note low specificity).
- P 15683252
  — *"which coordinate with two Zn(II) ions to form an unusual topology of two C4-type zinc fingers..."* (confirms zinc role belongs to DnaJ).
- P 8662861
  — *"two Zn(II) ions interact with each monomer of DnaJ."* (DnaJ stoichiometry).
- **Suggested curator questions:** (1) Is a single whole-proteome Zn-blot sufficient IDA support for a core MF given contradicting sequence/structure? (2) Should the DnaK/DnaJ shared reference trigger a carry-over review?
- **Suggested experiment:** purified-DnaK ICP-MS zinc stoichiometry with Cys15 mutant control.
- **Positive terms to keep:** ATP binding, ATP hydrolysis activity, unfolded protein binding, ATP-dependent protein folding chaperone.

Artifacts (computed provenance): `artifacts/evidence_matrix.csv`, `artifacts/hsp70_cysteine_census.csv`.

Provenance: All computed results (UniProt feature/GO parse, cysteine census, RCSB zinc-ligand query returning 0/63, QuickGO annotation provenance) were generated by executed code in Iteration 1; primary literature accessed via PubMed. No results were fabricated; the RCSB query returned HTTP 204 (empty) for DnaK-with-zinc.