NQO2 (P16083) — Function-Assignment Hypothesis Review

Hypothesis: NQO2 has NAD(P)H dehydrogenase (quinone) activity (GO:0003955). **Focus:** function_assignment · existing IBA annotation (GO_REF:0000033) · slug `function-hypothesis-go-0003955` **Gene:** human NQO2 / UniProt P16083

Executive Judgment

Verdict: Over-annotated → recommend REMOVAL of the IBA GO:0003955 (activity real, but this specific term is substrate-incorrect and redundant).

NQO2 is unequivocally a flavin-dependent, two-electron **quinone reductase**, so the *quinone-reductase* concept is correct. But the specific term **GO:0003955 "NAD(P)H dehydrogenase (quinone) activity"** (reaction: NAD(P)H + quinone → NAD(P)$^+$ + quinol) makes a **cofactor/substrate claim that NQO2 does not satisfy**. The definitive enzymology (Wu et al., 1997, P 9367528) shows NQO2 "uses dihydronicotinamide riboside (NRH) **rather than** NAD(P)H as an electron donor." UniProt (P16083) codifies this as **EC 1.10.5.1**; the NAD(P)H reaction (EC 1.6.5.2) belongs to the paralog **NQO1** (P15559).

Two ontology facts (verified via QuickGO this iteration) make the recommendation **removal rather than generalization**: 1. The biochemically exact term **GO:0001512 "dihydronicotinamide riboside quinone reductase activity"** (NRH + quinone → nicotinamide riboside + hydroquinone) is **already annotated to NQO2 with experimental evidence — IDA,** P 18254726 (plus IEA GO_REF:0000120). 2. **GO:0003955 is NOT an is_a ancestor of GO:0001512.** They are siblings in different oxidoreductase subtrees, so GO:0003955 cannot be defended as a merely-less-specific but still-true parent. It is a distinct, incorrect molecular function.

The IBA is therefore a **paralog over-annotation** (GO:0003955 native to NQO1, propagated across the shared PANTHER family via GO_REF:0000033), and it is both wrong on substrate and redundant with the correct experimental term.

Most important caveat: Do not delete NQO2's reductase function from the model — it is captured accurately by GO:0001512. The action is limited to the mis-specified IBA row.

Evidence Matrix

Citation	Evidence type	Stance	Claim tested	Key finding	Context
P 9367528 (Wu et al., 1997)	Direct assay (purified enzyme)	**Refutes cofactor**	Does NQO2 use NAD(P)H?	"NQO2 uses dihydronicotinamide riboside (NRH) **rather than** NAD(P)H as an electron donor"; FAD dimer; 2-e^- quinone reduction; dicoumarol-resistant	Recomb human
P 18254726 (Calamini et al., 2008)	Direct assay + X-ray structure	**Supports correct term**	NQO2 activity/ structure & ligands	Kinetic/thermodynamic/X-ray characterization of QR2; source of NQO2 **IDA GO:0001512**, FAD binding, Zn^{2+} binding, melatonin binding	Human crystal
P 10945627 (Knox et al., 2000; context ref)	Direct assay	**Qualifies (co-substrate)**	NQO2 oxidoreductase mechanism	CB1954 bioactivation by NQO2 is **co-substrate (NRH-analog)-mediated**; IDA source for GO:0016491/0016661/0009055	Human
UniProt P16083 (curated)	Database	**Qualifies**	Correct EC & reaction	**EC 1.10.5.1**; reaction NRH + quinone → nicotinamide riboside + quinol; cofactors FAD, Zn^{2+}; 231 aa	Human
UniProt P15559 (NQO1)	Database (paralog)	**Competing/ explanatory**	Which enzyme owns GO:0003955?	NQO1 = **EC 1.6.5.2**, NADH/ NADPH reactions, 274 aa	Human
QuickGO ontology (this run)	Computational (ontology)	**Qualifies**	Is GO:0003955 a valid parent of the true term?	GO:0001512 is_a ancestors = GO:0016679→GO:0016491; **GO:0003955 not an ancestor** (sibling, not parent)	GO relea
QuickGO annotation (this run)	Database	**Supports removal**	Is the correct term already present?	NQO2 already has **GO:0001512 (IDA,** P 18254726 **; IEA)**; GO:0003955 present	UniProt

Citation	Evidence type	Stance	Claim tested	Key finding	Context
				only as **IBA (GO_REF:0000033)**	
P 18996184 (Gaikwad et al., 2009)	Direct assay	**Supports reductase core**	Does NQO2 reduce quinones?	NQO2 reduces estrogen o-quinones using an NRH-type cofactor (BNAH); faster than NQO1	Human recombi
P 21506232 (Dufour et al., 2011)	Structural/ inhibitor	**Qualifies (flavoprotein)**	Mechanism & FAD	FAD flavoprotein; inhibitors alkylate flavin; NQO1-distinct selectivity	X-ray + I

GO Curation Implications

Lead (requires curator verification):

- **Molecular Function — REMOVE the IBA GO:0003955 from NQO2** (or mark NOT / do-not-propagate). It asserts NAD(P)H substrate that NQO2 cannot use and is a paralog carry-over from NQO1.
- **Retain the already-present GO:0001512** "dihydronicotinamide riboside quinone reductase activity" (IDA, P 18254726) as the accurate leaf MF term. No new term needs to be created.
- Because GO:0003955 is a **sibling (not ancestor)** of GO:0001512, keeping it is not a harmless generalization — it introduces a false substrate assertion into the model.
- Broader true ancestors already annotated (GO:0016491 oxidoreductase activity; GO:0016661 acting on other nitrogenous donors; GO:0009055 electron transfer activity; GO:0048038 quinone binding; GO:0071949 FAD binding; GO:0008270 zinc ion binding) remain valid. Avoid defaulting to generic "protein binding."

GO decision table

Term	Current on NQO2	Recommended action	Basis
GO:0003955 NAD(P)H dehydrogenase (quinone) activity	**IBA** (GO_REF:0000033)	**Remove / NOT** — substrate-incorrect, paralog carry-over, non-ancestral to true term	P 9367528; UniProt EC 1.10.5.1; QuickGO ancestry
GO:0001512 dihydronicotinamide riboside quinone reductase activity	**IDA** (P 18254726) + IEA	**Retain** as accurate MF leaf term	P 9367528; P 18254726; EC 1.10.5.1

NQO2 (P16083) GO MF decision: REMOVE IBA GO:0003955 (NAD(P)H), RETAIN GO:0001512 (NRH) | structural basis: NQO1 has 43 extra C-term residues

GO_ID	Label	Evidence	Reference	Action	Rationale
GO:0003955	NAD(P)H dehydrogenase (quinon	IBA	GO_REF:0000033	REMOVE / NOT	Substrate-incorrect (NQO2 uses NF
GO:0001512	dihydronicotinamide riboside qui	IDA(+IEA)	PMID:18254726	RETAIN	Matches NQO2 EC 1.10.5.1; alread

Figure 1. *GO molecular-function decision table for NQO2 (P16083). GO:0003955 "NAD(P)H dehydrogenase (quinone) activity" (IBA, GO_REF:0000033) is substrate-incorrect (NQO2 uses NRH, not NAD(P)H;* P *9367528* *) and redundant with the already-annotated, experimentally-supported GO:0001512 "dihydronicotinamide riboside quinone reductase activity" (IDA,* P *18254726* *). Recommended action: remove GO:0003955; retain GO:0001512.*

Mechanistic Scope

- **Immediate molecular function tested:** FAD-mediated **two-electron reduction of quinones to hydroquinones**, electrons supplied by a **dihydronicotinamide riboside (NRH)** donor in a ping-pong mechanism; Zn^{2+} is structural.
- **Correctly in scope:** quinone reduction (menadione and other quinones, estrogen o-quinones, vitamin K quinones), NRH-dependent nitroreduction (CB1954). Captured by GO:0001512.

- **Out of scope for this MF term (BP/CC or ligand-binding):** neuronal "memory constraint"/ metabolic-buffer role (Rosenblum lab), Alzheimer's phenotype modulation, melatonin MT3 binding-site identity (GO:1904408) and resveratrol binding (GO:1905594). These are downstream physiology or ligand properties, not the electron-donor chemistry adjudicated here.

Conflicts and Alternatives

- **Paralog confusion (primary):** GO:0003955 is correct for **NQO1** (EC 1.6.5.2, NADH/NADPH). Its presence on NQO2 is explained by IBA propagation, not NQO2 biochemistry. Structural basis: NQO2 (231 aa) lacks the ~43-residue C-terminal region present in NQO1 (274 aa) that helps form the adenosine/2′-phosphate NAD(P)H-binding site, so NQO2 uses the smaller NRH.
- **In-vitro cofactor surrogates:** NQO2 assays use synthetic donors (BNAH; EP-0152R) because free NRH is not a standard bulk metabolite; consistent with EC 1.10.5.1, not with NAD(P)H use.
- **No credible primary report** shows efficient NAD(P)H-driven catalysis by NQO2.
- **Self-correction:** Iteration 1 tentatively named the replacement term GO:0033856; that ID is actually *pyridoxine 5′-phosphate synthase activity*. The correct term is **GO:0001512**, confirmed via QuickGO.

Knowledge Gaps

1. **Physiological NRH source in vivo.** Checked: literature uses in-vitro NRH/BNAH surrogates. Matters for BP context, not the MF term. Resolve via tissue metabolomics for NRH and NRH-generating enzymes.
2. **Whether the review pipeline treats a non-ancestral IBA as "generalize" vs "remove."** Checked ancestry (GO:0003955 not ancestor of GO:0001512), which argues for removal. Curator should confirm project policy.
3. **Quantitative NAD(P)H vs NRH kinetics.** Existing data are qualitative (NRH-preference). A kcat/Km ratio would formally bound the error; confirmatory only.

Discriminating Tests

- **Side-by-side kinetics:** NQO2 quinone reduction with NRH/BNAH vs NADH vs NADPH → expect robust NRH activity, negligible NAD(P)H (discriminates GO:0001512 from GO:0003955).
- **Structure/domain check:** confirm absence of the NQO1 C-terminal NAD(P)H subdomain in NQO2 (PDB 1QR2 vs NQO1 structures; sequence alignment).
- **Chimera/mutagenesis:** graft the NQO1 C-terminal extension onto NQO2 to test gain of NAD(P)H use — causal test of the domain–cofactor link.

Curation Leads (require curator verification)

- **Candidate action:** Remove/NOT the IBA **GO:0003955** on NQO2; retain existing IDA **GO:0001512**.
- **Candidate reference to verify (donor specificity):**

P 9367528

— *"NQO2 uses dihydronicotinamide riboside (NRH) rather than NAD(P)H as an electron donor. It catalyzes a two-electron reduction of quinones and oxidation-reduction dyes."*

- **Candidate reference supporting the correct term (already the IDA source):**

P 18254726

(Calamini et al., 2008, QR2 kinetics + X-ray) — verify it is the IDA basis for GO:0001512.

- **Context reference:**

P 10945627

(CB1954 co-substrate-mediated bioactivation) confirms NRH-type co-substrate, not NAD(P)H.

- **Database support:** UniProt P16083 (EC 1.10.5.1, NRH→quinol reaction, FAD+Zn^{2+}); QuickGO ancestry showing GO:0003955 is not an ancestor of GO:0001512.
- **Suggested curator question:** Does project policy remove a substrate-incorrect, non-ancestral IBA term when the correct experimental term is already present, or retain it flagged?

- **Suggested experiment:** NRH-vs-NAD(P)H kinetic comparison to document cofactor discrimination quantitatively.

Provenance (executed this review)

- UniProt REST: NQO2 P16083 = 231 aa, EC 1.10.5.1, NRH→quinol, FAD+Zn^{2+}; NQO1 P15559 = 274 aa, EC 1.6.5.2, NAD(H)/NADP(H). Confirms paralog cofactor divergence + 43-aa length difference.
- QuickGO ontology: GO:0001512 is_a ancestors = {GO:0016679, GO:0016491, GO:0003824, GO:0003674}; GO:0003955 NOT among them (sibling relationship).
- QuickGO annotation (UniProtKB:P16083, MF): GO:0001512 = IDA (P 18254726) + IEA; GO:0003955 = IBA only (GO_REF:0000033).
- NCBI eSummary:

  P 18254726

  = QR2 melatonin kinetics/X-ray;

  P 10945627

  = CB1954 co-substrate bioactivation.
- Sequence comparison (UniProt FASTA, computed): NQO2 231 aa vs NQO1 274 aa; NQO1 carries exactly **43 extra C-terminal residues** (…NFQAGFLMKKEVQDEEKNKKFGLSVGHHLGKSIPTDNQIKARK) absent in NQO2 — the C-terminal segment forming part of the NAD(P)H adenosine-binding site, i.e. the structural reason NQO2 cannot use NAD(P)H.
- Literature:

  P 9367528

  (definitive), 18996184, 21506232, plus review 18374191.

Artifacts generated

- `NQO2_GO_decision_table.png` — rendered GO MF decision table (remove IBA GO:0003955; retain GO:0001512), auto-saved during execution.

- Evidence matrix and GO decision table are embedded above as artifact-friendly Markdown tables (the code executor sandbox could not write CSVs to the job directory; the executed code and its printed outputs above are the provenance).
