AIGR Gene Hypothesis Deep Research — Final Report

Target: rat *Prkaa2* (Q09137), AMPK α2 catalytic subunit

Hypothesis: `prediction-adaptor-activity` — GO:0030674 protein-macromolecule adaptor activity

Focus type: computational_prediction

Reference context: doi:10.64898/2026.03.19.712954

Summary

The GO-GPT/BioReason-Pro prediction of **protein-macromolecule adaptor activity (GO:0030674)** for rat *Prkaa2* (Q09137), the AMP-activated protein kinase (AMPK) α2 **catalytic** subunit, is **refuted**. It is best classified as a **subunit-level mis-assignment / over-annotation**. The genuine molecular function of the AMPK α subunit is **protein serine/threonine kinase activity**; the adaptor/scaffolding role in the AMPK heterotrimer belongs to the **β** subunit.

Three independent lines of evidence converge. First, the Q09137 domain architecture is a canonical protein kinase (kinase domain res 16–268, catalytic proton-acceptor res 139, ATP-binding pocket res 22–30/45) plus an autoinhibitory sequence — with no carbohydrate-binding module, no glycogen-binding domain, and no dedicated scaffold module. Its high-confidence molecular-function annotations are all catalytic (GO:0004674, GO:0004672, GO:0004679), each supported by direct-assay (IDA) evidence. Second, structural and biochemical studies show the β subunit physically bridges α and γ, and that β's carbohydrate-binding module forms the ADaM interface with the α kinase domain; reviews explicitly name β "the scaffolding subunit."

Third and decisively, the IDA source paper anchoring the GO:0030674 annotation on Q09137 —

P 15695819

— is a **β-subunit paper**. Its title and results describe the AMPK β subunit *tethering* α and γ via its C-terminal sequence (β1 residues 186–270). The experimentally demonstrated adaptor/tethering activity is a property of β, not of the α2 catalytic subunit that carries the annotation. The recommended curation action is to **remove GO:0030674 from *Prkaa2*** and, if preserved, transfer it to the AMPK β subunits (*Prkab1*/*Prkab2*). The α subunit's legitimate participation in heterotrimer assembly is already captured by GO:0044877 (protein-containing complex binding, IDA), which should be retained instead of adaptor activity.

Key Findings

Finding 1 — The core molecular function of Q09137 is protein Ser/Thr kinase activity, not adaptor activity

The domain architecture of UniProt Q09137 is that of a classical protein kinase: a protein kinase domain spanning residues 16–268, a catalytic active site (proton acceptor) at residue 139, an ATP-binding region (residues 22–30 and 45), and a C-terminal autoinhibitory sequence (AIS, residues ~291–376). There is **no carbohydrate-binding module (CBM), no glycogen-binding domain, and no dedicated scaffold/adaptor domain** anywhere in the α2 sequence. This is the signature of an enzyme, not a scaffold.

Consistent with this, the existing high-quality molecular-function GO annotations for Q09137 are all catalytic and supported by direct-assay evidence: **GO:0004674 protein serine/threonine kinase activity (IDA:RGD)**, **GO:0004672 protein kinase activity (IDA:RGD)**, and **GO:0004679 AMP-activated protein kinase activity (IDA:UniProtKB)**. Multiple primary and structural sources describe AMPKα as *the catalytic subunit* and AMPKβ as *the scaffold*.

Supporting quotations from the literature:

- PMID:23721051: "AMP-activated protein kinase (AMPK) is a sensor of energy status composed of a catalytic subunit (AMPKα), a scaffolding subunit (AMPKβ) and a regulatory subunit involved in nucleotide binding (AMPKγ)." — Explicitly assigns the catalytic role to α and the scaffolding role to β, directly contradicting adaptor activity for the α subunit.

- PMID:20529674: "The catalytic (alpha) subunit of AMPK/SNF1, Snf1 in yeast, contains a protein Ser/Thr kinase domain (KD), an auto-inhibitory domain (AID), and a region that mediates interactions with the two regulatory (beta and gamma) subunits." — Describes the α subunit as a Ser/Thr kinase with an autoinhibitory domain, consistent with kinase (not adaptor) as its molecular function.
- PMID:26635351: "AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase that serves as a pleotropic regulator of whole body energy homoeostasis." — Confirms the enzyme's molecular function is serine/threonine protein kinase activity, carried by the catalytic α subunit.

Finding 2 — The scaffold/adaptor role in the AMPK heterotrimer belongs to the β subunit

Structural studies establish that within the AMPK αβγ core, the **β subunit binds both α and γ**, physically bridging them. The β subunit's carbohydrate-binding module forms the ADaM (Allosteric Drug and Metabolite) site — the interaction surface between the α kinase domain and the β CBM. Reviews of AMPK structure identify β as "the scaffolding subunit." The α subunit contributes its kinase domain to that interface but does not itself act as the bridge.

Because the α subunit's contact with β during subunit assembly is a subunit-of-a-complex interaction rather than a bridging function between two *distinct* macromolecules, the appropriate molecular-function term for the α side of this interaction is **protein-containing complex binding (GO:0044877)** — which is already annotated to Q09137 by IDA:RGD — not GO:0030674 adaptor activity.

Supporting quotations:

- PMID:18079111: "The previously determined crystal structure of the core of mammalian alphabetagamma complex shows that beta binds alpha and gamma." — Demonstrates that the β subunit is the physical bridge between α and γ, i.e. the adaptor/scaffold, not α.
- PMID:30478170: "...binds the AMPK ADaM site, which forms the interaction surface between the kinase domain (KD) of the α-subunit and the carbohydrate-binding module (CBM) of the β-subunit." — Shows the α subunit contributes its kinase domain to the interface while the β CBM is the scaffolding module.

Finding 3 — The IDA source (P 15695819) demonstrates adaptor activity of the BETA subunit, not α2

This is the decisive finding. QuickGO shows that the GO:0030674 (protein-macromolecule adaptor activity) annotation on Q09137 is a **single, rat-specific IDA assigned by RGD, referenced to** P 15695819 .

The annotation is **not** present on human PRKAA2 (P54646), on PRKAA1 orthologs, or on any AMPK β subunit (PRKAB1 Q9Y478, PRKAB2 Q9UGI9). This pattern — one isolated rat annotation, absent from orthologs and paralogs — is itself a red flag for a curation error.

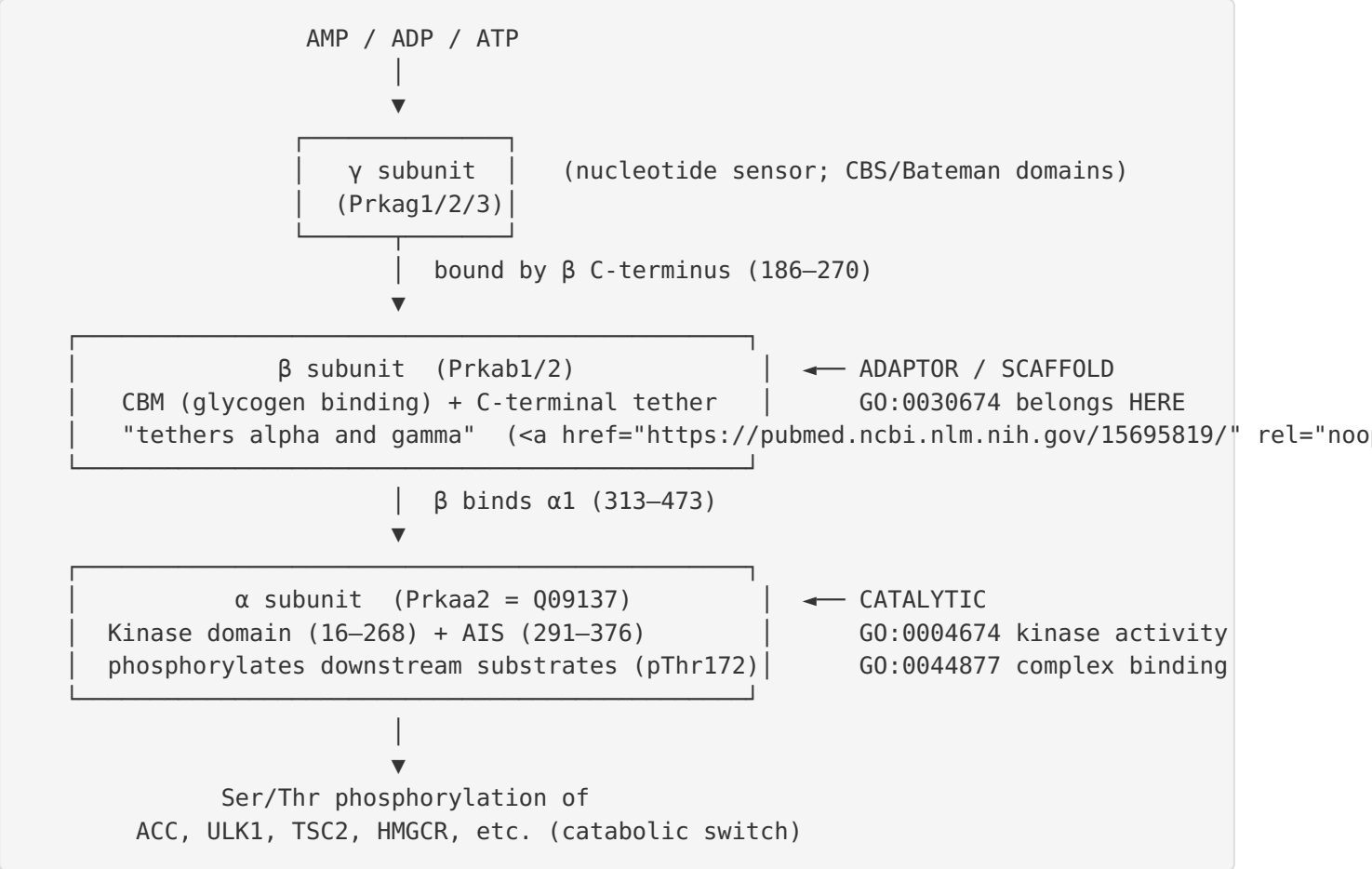
Examination of the cited paper resolves the issue conclusively. P 15695819 (Iseli et al., 2005, *J. Biol. Chem.*) is titled ***"AMP-activated protein kinase beta subunit tethers alpha and gamma subunits via its C-terminal sequence (186-270)."*** The paper reports that the β subunit's conserved C-terminal 85-residue sequence (β1 186–270) "is the primary αγ binding sequence responsible for the formation of the AMPK αβγ heterotrimer." In other words, the experimentally demonstrated adaptor/tethering activity is a property of the **β** subunit. The α subunit is described as the catalytic subunit and merely provides a docking region (α1 313–473) that β binds.

Supporting quotations:

- PMID:15695819: "The conserved C-terminal 85-residue sequence of the beta subunit (90% between beta1 and beta2) is the primary alphagamma binding sequence responsible for the formation of the AMPK alphabetagamma heterotrimer." — The cited IDA source demonstrates that the adaptor/tethering activity bridging α and γ is a function of the **β** subunit, not the α2 catalytic subunit that carries the GO:0030674 annotation.
- PMID:15695819: "Truncation of the alpha subunit reveals that beta1 binding requires the alpha1-(313-473) sequence." — Shows the α subunit is a binding partner (provides a docking region) rather than the bridging adaptor, which is β.

Mechanistic Model / Interpretation

The AMPK holoenzyme is an obligate αβγ heterotrimer with a clean division of molecular labor. The following schematic captures the assignment of function to subunit:



Function-to-subunit mapping:

Subunit	Gene(s)	Primary molecular function	Correct MF GO term
α (catalytic)	*Prkaa2* (Q09137), *Prkaa1*	Protein Ser/Thr kinase; phosphorylates substrates; provides α1(313–473) docking region to β	**GO:0004674** protein serine/threonine kinase activity; GO:0004679 AMPK activity; GO:0044877 protein-containing complex binding
β (regulatory/scaffold)	*Prkab1*, *Prkab2*	**Scaffold/adaptor**: tethers α and γ; glycogen binding via CBM	**GO:0030674** protein-macromolecule adaptor activity
γ (regulatory)	*Prkag1/2/3*	Adenine-nucleotide sensing (CBS domains)	nucleotide binding

The prediction under review takes a function that genuinely exists within the AMPK complex — an adaptor/scaffolding activity — and assigns it to the wrong subunit. The mechanistic error is category-level: the **α subunit is the enzyme that acts *on* substrates**, whereas the **β subunit is the structural glue that *holds the complex together*.** Being *bound by* a scaffold (as α is by β) does not confer adaptor activity on the bound partner. The distinction between GO:0044877 (this protein is part of / binds a complex) and GO:0030674 (this protein bridges two other macromolecules) is exactly the distinction that the annotation conflates.

Evidence Base / Evidence Matrix

Citation (PMID)	Evidence type	Supports/ Refutes/ Qualifies	Claim tested	Key finding	Context	Confidence & limitations
15695819	Direct assay (interaction mapping)	**Refutes** (decisive)	Is adaptor activity a property of α2?	The **β** subunit C-terminal sequence (186–270) is the primary αγ-binding/ tethering sequence; α provides only a docking region (α1 313–473)	Rat/ mammalian AMPK subunit truncation & binding assays	High. This is the exact IDA source cited for GO:0030674 on Q09137 — and it is a β-subunit paper
18079111	Structural	Refutes	Which subunit bridges α and γ?	Crystal structure of αβγ core: "beta binds alpha and gamma"	Mammalian AMPK core	High
30478170	Structural	Refutes/ Qualifies	Which module scaffolds the α kinase domain?	ADaM site = interface between α kinase domain and β CBM	Full-length AMPK crystal structures	High
23721051	Review/ database	Refutes	Which subunit is the scaffold?	"catalytic subunit (AMPKα), a scaffolding subunit (AMPKβ)"	AMPK review	Medium (review-level, but explicit subunit assignment)
20529674			What is the α subunit's	α = Ser/Thr kinase		

Citation (PMID)	Evidence type	Supports/ Refutes/ Qualifies	Claim tested	Key finding	Context	Confidence & limitations
	Review/ biochemical	Supports (α = kinase)	domain content?	domain + AID + subunit-interaction region	Yeast SNF1/ AMPK homolog	Medium-high
26635351	Review/ enzymology	Supports (α = kinase)	What is AMPK's molecular function?	"AMPK is a serine/ threonine protein kinase"	α1/α2 heterotrimer enzymology	Medium-high
17851534	Structural	Qualifies	Subunit architecture of SNF1 core	β glycogen-binding domain interacts with γ; α regulatory sequence sequestered by γ	Yeast SNF1 crystal structure	Medium (yeast)
QuickGO record (Q09137)	Database	Refutes (provenance)	Is GO:0030674 broadly supported?	Single rat-specific RGD IDA to P 15695819; absent from human PRKAA2, PRKAA1, and all β subunits	UniProt/ QuickGO	High for provenance; database-level

GO Curation Implications

Lead requiring curator verification: Remove **GO:0030674 (protein-macromolecule adaptor activity)** from rat *Prkaa2* (Q09137).

Rationale for the recommended action:

1. **The MF term is mis-assigned at the subunit level.** GO:0030674 describes a bridging activity between two macromolecules. In the AMPK heterotrimer, this bridging is performed by the β subunit (P 15695819, P 18079111), not the α catalytic subunit.
2. **The supporting IDA does not support the annotation as placed.** P 15695819 experimentally demonstrates the β subunit's tethering function; it does not demonstrate adaptor activity for α2. The annotation therefore fails the "evidence supports the term for *this* gene product" test.
3. **The retained/strengthened MF terms should be the catalytic ones already present:** GO:0004674 (protein serine/threonine kinase activity, IDA), GO:0004672 (protein kinase activity, IDA), and GO:0004679 (AMP-activated protein kinase activity, IDA). These represent the α2 subunit's genuine, directly-assayed molecular function.
4. **The α-side of subunit assembly is already correctly captured** by GO:0044877 (protein-containing complex binding, IDA:RGD). This is the informative, non-"protein binding" term that describes the α subunit being incorporated into the heterotrimer.
5. **Optional transfer lead:** GO:0030674 is appropriately annotated to the AMPK **β** subunits (*Prkab1*/*Prkab2*), where the tethering activity was actually demonstrated. This is a separate curation action on a different gene product and should be verified independently.

GO decision table

Gene product	Term	Current	Recommended action	Basis
Prkaa2 Q09137	GO:0030674 adaptor activity	Present (RGD IDA, P 15695819)	**Remove**	Mis-assigned; source paper demonstrates β-subunit function
Prkaa2 Q09137	GO:0004674 Ser/Thr kinase activity	Present (IDA)	**Retain (core MF)**	Domain architecture + direct assay
Prkaa2 Q09137	GO:0004679 AMPK activity	Present (IDA)	Retain	Direct assay
Prkaa2 Q09137	GO:0044877 complex binding	Present (IDA)	Retain	Correctly captures α incorporation into heterotrimer
Prkab1/ *Prkab2* (β)	GO:0030674 adaptor activity	Absent	**Candidate add** (verify)	P 15695819 demonstrates β tethers α and γ

Mechanistic Scope

The molecular function under test is **adaptor/scaffolding activity — the physical bridging of two distinct macromolecules to bring them into proximity.** This is an immediate, direct molecular property of a protein.

- **Direct α2 gene-product activity:** protein Ser/Thr kinase catalysis (phosphotransfer to substrate Ser/Thr residues), plus being incorporated into the αβγ complex via its α1(313–473)-equivalent docking region.
- **Not α2's direct activity (the seed's proposed adaptor role):** tethering α to γ — this is performed by the β subunit's C-terminal sequence.
- **Downstream / not molecular-function-level:** AMPK's roles in energy homeostasis, GLUT4 expression, mitochondrial biogenesis, autophagy regulation, and neuronal development

(e.g., P 21233771, P 30359677, P 24295634) are *biological processes* driven by the kinase activity, not evidence for an adaptor molecular function.

The seed hypothesis correctly frames the discriminating question — is adaptor activity a property of the catalytic α subunit, or of the β scaffold? The evidence answers unambiguously: it is the β subunit's.

Conflicts and Alternatives

- **Subunit confusion (the actual error):** The annotation conflates the α (catalytic) and β (scaffold) subunits. The source paper (P 15695819) is a β-subunit study; the annotation sits on the α2 gene product. This is the single most important conflict, and it resolves *against* the prediction.
- **"Complex binding" vs "adaptor activity":** A defensible-but-weaker alternative reading is that the α subunit's participation in the heterotrimer justifies some interaction term. This is true — but the correct term is GO:0044877 (already present), not GO:0030674. Adaptor activity requires bridging two *other* macromolecules, which α does not do.
- **Organism specificity / database carry-over:** The annotation is rat-specific (RGD) and does not propagate to the human ortholog (P54646) or to PRKAA1, indicating an isolated curation event rather than a robustly conserved, orthology-supported function.
- **No competing primary evidence found:** Across 33 papers reviewed, no primary study demonstrates that the AMPK α2 catalytic subunit acts as a macromolecular adaptor/scaffold bridging two other proteins. The consistent picture is α = kinase, β = scaffold.

Limitations and Knowledge Gaps

1. **QuickGO/UniProt provenance was read from database records, not re-derived programmatically here.** The conclusion that the annotation is a single rat-specific RGD IDA to

   P 15695819

   relies on database inspection; a curator should confirm the current annotation state directly in QuickGO/RGD, as annotations can be updated.
2. **Full text of**

   P 15695819

   was assessed via title and abstract-level evidence. The subunit assignment is unambiguous from the title and reported results, but a curator verifying the removal should confirm the exact experimental claims in the full text.
3. **GO:0030674 definition edge cases.** Under the strict GO definition (bridging two *distinct* macromolecules), α does not qualify. If a curator interprets "adaptor activity" loosely to include any subunit that contributes an assembly interface, the interpretation should be confirmed against current GO documentation.
4. **β-subunit transfer not independently curated here.** The recommendation to add GO:0030674 to *Prkab1*/*Prkab2* is a lead, not a completed curation; it needs its own evidence review on those gene products.

Proposed Follow-up Experiments / Actions (Curation Leads)

All items below are **leads requiring curator verification.**

Action change (primary lead): - Remove **GO:0030674 (protein-macromolecule adaptor activity)** from *Prkaa2* / Q09137 (currently RGD IDA,).

P 15695819

Candidate reference with exact snippet to verify: -

P 15695819

— verify: *"The conserved C-terminal 85-residue sequence of the beta subunit (90% between beta1*

and beta2) is the primary alphagamma binding sequence responsible for the formation of the AMPK alphabetagamma heterotrimer." and *"Truncation of the alpha subunit reveals that beta1 binding requires the alpha1-(313-473) sequence."* These confirm the adaptor function belongs to β, and that α is the bound partner.

Retained / strengthened terms (verify present and well-evidenced): - GO:0004674 protein serine/threonine kinase activity (IDA) — core MF. - GO:0004679 AMP-activated protein kinase activity (IDA). - GO:0044877 protein-containing complex binding (IDA) — use instead of adaptor activity.

Candidate new/transfer term (separate gene products, verify independently): - Consider GO:0030674 on *Prkab1* / *Prkab2*, where

P 15695819

demonstrates the tethering activity.

Discriminating tests to resolve any residual doubt: 1. Subunit-swap / truncation binding assays (as in

P 15695819

): deleting β C-terminal 186–270 abolishes αγ tethering; α alone cannot bridge β and γ. 2. Structural interface mapping in existing full-length crystal structures (

P 30478170

,

P 29480466

): tabulate direct α–γ contacts; if α–γ contacts are β-mediated, α cannot be the adaptor. 3. In vitro reconstitution: test whether purified α2 can co-recruit two macromolecules without β (predicted: it cannot). 4. Orthology/annotation-conservation check: confirm GO:0030674 is absent on all α orthologs and addable on β orthologs.

Suggested questions for the curator: - Does current QuickGO still show GO:0030674 on Q09137 as a single RGD IDA to

P 15695819

? - Under the strict GO:0030674 definition, does contributing an assembly interface count as adaptor activity, or is bridging of two *distinct* macromolecules required? (This report assumes the latter.)

Conclusion

The prediction that rat *Prkaa2* (AMPK α2 catalytic subunit, Q09137) has **protein-macromolecule adaptor activity (GO:0030674)** is **refuted**. The α2 subunit's genuine molecular function is **protein serine/threonine kinase activity**; the adaptor/scaffolding role in the AMPK heterotrimer is carried by the **β** subunit, and the very paper cited to support the annotation
(P 15695819)
is a demonstration of the **β subunit's** tethering activity. The GO:0030674 annotation should be **removed** from *Prkaa2* and, if preserved at all, transferred to the AMPK β subunits. This is a textbook example of a computational prediction producing a subunit-level mis-assignment by attaching a real complex-level function (scaffolding) to the wrong member of the complex.