DISTILLATION №83 · ALEMBIC LABS

MOTS-C — PRO-12 → A-AMINOISOBUTYRIC ACID (AIB) SINGLE SUBSTITUTION AT THE CENTRAL YPR HINGE TO REMOVE THE PROLINE KINK AND LOCALLY PROMOTE HELICAL GEOMETRY ADJACENT TO THE CATIONIC C-TERMINAL PATCH

generated 2026-05-05T06:34:49.924774+00:00

DISCARDED LONGEVITY

PRO-12 → A-AMINOISOBUTYRIC ACID (AIB) SINGLE SUBSTITUTION AT THE CENTRAL YPR HINGE TO REMOVE THE PROLINE KINK AND LOCALLY PROMOTE HELICAL GEOMETRY ADJACENT TO THE CATIONIC C-TERMINAL PATCH

5'-AMP-ACTIVATED PROTEIN KINASE CATALYTIC SUBUNIT ALPHA-2

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
61.9%	0.550 / 0.179	DISCARDED

TARGET	UNIPROT	BINDING PROBABILITY
5'-AMP-activated protein kinase catalytic subunit alpha-2	P54646	—

TLDR

DISTILLATION №83 tested whether replacing Pro-12 of MOTS-c with α-aminoisobutyric acid (Aib) could remove the proline kink at the YPR hinge and allow the C-terminal RKLR cationic patch to project as a coherent helical face onto the AMPK α2 catalytic subunit. The structural predictor returned a monomer-level pLDDT of 0.62 — consistent with prior MOTS-c folds — but an interface score of ipTM

0.18, indicating no convergent docking pose was found. This fold is DISCARDED as a tool-limit result: Boltz-2 could not adjudicate the interface, not because the hypothesis is biologically disproved. Separately, the literature reveals the dominant mechanistic model positions MOTS-c as an indirect AMPK activator via AICAR rather than a direct AMPK α2 ligand, which adds an independent biological complication the structural predictor cannot resolve.

EXECUTIVE SUMMARY

DISTILLATION №83 tested Pro-12 → Aib rigidification of MOTS-c's YPR hinge to align the RKLR cationic patch for AMPK α2 engagement. ipTM 0.18 — predictor non-convergence at the interface, not biological disproof. Direct MOTS-c–AMPK α2 binding remains experimentally unvalidated.

DETAILED ANALYSIS

MOTS-c is a 16-residue mitochondrial-derived peptide (MDP) encoded in the 12S rRNA locus of the mitochondrial genome, first characterized by Lee et al. (2015) as a regulator of insulin sensitivity and metabolic homeostasis. Its canonical mechanism involves disruption of the intracellular folate-methionine cycle in skeletal muscle, accumulation of AICAR, and downstream AMPK activation via AMP-mimicry at the regulatory γ subunit — an indirect route that does not require the peptide to physically dock onto the AMPK α2 catalytic domain. Nonetheless, a separate line of evidence (PMID:39321430) demonstrates MOTS-c is capable of direct protein-protein interactions (LARS1 binding), leaving open whether a direct AMPK α2 engagement mode exists in parallel.

The hypothesis for this fold was mechanistically clean and chemically grounded: Pro-12 sits at the YPR hinge, one residue upstream of the cationic RKLR patch (Arg-13/Lys-14/Arg-16) that prior lab folds — particularly DISTILLATION №19 (K13R) — identified as the likely AMPK-engaging face. Proline is a canonical helix breaker; replacing it with Aib, whose gem-dimethyl Cα locks φ/ψ into helical/310 values, is the textbook approach to straightening such a kink. The same Aib strategy has been applied to GLP-1, GHRH, and parathyroid hormone analogs. If the RKLR patch does engage AMPK α2 electrostatically, allowing those residues to project coherently from an α-helical scaffold should, in principle, increase binding complementarity.

The structural prediction produced a peptide monomer pLDDT of 0.618 — essentially identical to the 0.62 baseline seen across MOTS-c folds #19, #43, and #71 — indicating Boltz-2's confidence in the peptide's internal fold is typical for this sequence class and length. The critical failure is at the interface: ipTM of 0.179 is well below any threshold for confident docking prediction. Boltz-2 did not converge on a stable, specific pose between the Aib-12 peptide and the AMPK α2 catalytic surface. The pTM of 0.550 reflects a system-level uncertainty that is equally

uninformative. Whether the Aib substitution achieved its intended local conformational goal — straightening the YPR hinge — cannot be read from these numbers; pLDDT is a per-residue confidence metric, not a secondary structure reporter.

This discard is therefore a tool-limit result rather than a biological invalidation. Boltz-2 is not equipped to resolve sub-nanomolar or allosteric peptide interfaces with confidence at this size regime, especially for peptides lacking a co-crystal template at the target. The same structural predictor discarded the hydrocarbon-stapled variant of MOTS-c (fold #30) for closely related reasons — low ipTM despite plausible chemistry — and that fold's discard likewise could not be interpreted as evidence that stapling fails for MOTS-c.

The literature layer adds a genuinely important biological complication that sits above the tool-limit issue: if MOTS-c activates AMPK exclusively via AICAR production (indirect mechanism), then improving the helical geometry of the C-terminal RKLR patch would not alter AMPK activation at all — the mechanism simply does not involve the peptide contacting AMPK α2. The direct-binding hypothesis is plausible and novel but lacks any published structural or biophysical support. This is not a reason to abandon the hypothesis; it is a reason to validate the binding interaction itself before pursuing analog optimization.

Heuristic sequence-based properties for the Aib-12 variant are modest: aggregation propensity 0.161 (low, favorable), stability score 0.319 (moderate), BBB penetration estimate 0.273 (low, expected for a 16-residue cationic peptide), and a half-life estimate in the moderate range (~30 min–2 h). These are not wet-lab measurements and carry the usual in silico caveats, but they do not flag new liabilities introduced by the Aib substitution relative to native MOTS-c.

Across the MOTS-c fold series, this lab has now explored C-terminal cationic patch optimization (fold #19), proteolytic stability at the GYIF junction (fold #43), PEGylation for half-life extension (fold #71), N-terminal lipidation for membrane association (fold #25), and hydrocarbon stapling of the central turn (fold #30). The Aib-12 fold is the first in this series to target backbone conformational rigidification at the YPR hinge specifically to improve AMPK engagement — a distinct rationale that remains worth pursuing with better tools. The convergent message from folds #30 and #83 is that docking-based predictions of MOTS-c–AMPK α2 interfaces are not currently resolvable by the structural predictors in this pipeline, and that biophysical validation of the direct binding interaction should precede further computational analog design.

RESEARCH BRIEF

DISTILLATION №83 — DISCARDED

MOTS-C PRO-12 → AIB SUBSTITUTION | AMPK A2 | YPR HINGE RIGIDIFICATION

TLDR

This fold was **DISCARDED** due to a **tool-limit failure at the protein-protein interface**: Boltz-2 returned an ipTM of 0.179 for the Aib-12 MOTS-c / AMPK α2 complex, indicating the structural predictor could not converge on a stable, specific docking pose. This is not a biological invalidation of the hypothesis. A parallel complication from the literature — that MOTS-c's dominant established mechanism of AMPK activation is indirect (via AICAR accumulation), not through direct binding to the AMPK α2 catalytic subunit — adds biological uncertainty that the structural predictor cannot resolve either way.

WHAT WE TRIED

MOTS-c (MRWQEMGYIFYPRKLR) carries a proline at position 12 that breaks the otherwise potentially helical C-terminal YPRKLR segment. Prior MOTS-c folds in this lab — particularly **DISTILLATION №19** (K13R substitution, PROMISING, pLDDT 0.63) — identified the Arg-13/Lys-14/Arg-16 cationic patch as the likely AMPK-engaging face. The hypothesis here was that replacing Pro-12 with α-aminoisobutyric acid (Aib) — a gem-dimethyl Cα residue that locks φ/ψ into helical values and is the canonical helix-nucleating non-natural amino acid in peptide chemistry — would allow the RKLR patch to project as a coherent helical face, increasing electrostatic complementarity with the acidic regulatory surface of AMPK α2.

This represents a distinct strategy from all prior MOTS-c folds: fold #19 optimized cationic patch character; fold #43 targeted proteolytic stability at the GYIF junction; fold #71 extended half-life via PEGylation; fold #25 explored membrane association via N-terminal myristoylation; fold #30 attempted helical pre-organization via an i,i+4 hydrocarbon staple across residues 5–9. The Aib-12 substitution is the first fold in this series focused on conformational rigidification at the YPR hinge specifically to improve AMPK interface geometry.

WHY IT WAS DISCARDED

Boltz-2 returned an **ipTM of 0.179** — far below any threshold for confident interface prediction (generally ≥ 0.5 for meaningful docking signal). The peptide monomer pLDDT of 0.618 is typical for MOTS-c folds in this pipeline (consistent with folds #19, #43, #71) and does not itself represent a failure, but the interface did not converge. No Chai-1 cross-validation was available, and the Boltz-2 affinity module produced no values.

This mirrors the outcome of **DISTILLATION №30**, where an all-hydrocarbon i,i+4 stapled MOTS-c variant was similarly discarded with pLDDT 0.60 and a failing ipTM against AMPK α2. The convergent pattern across folds #30 and #83 suggests that MOTS-c–AMPK α2 docking is currently below the resolution threshold of the structural predictors in this pipeline — likely because there is no deposited co-crystal structure to template against, and the peptide is short enough that Boltz-2 cannot confidently resolve a specific binding pose from sequence alone.

Additionally, the literature does not support a direct MOTS-c–AMPK α2 binding interaction: the mechanistic consensus (PMID:25738459, PMID:36677050, PMID:36761202) holds that MOTS-c activates AMPK via intracellular AICAR accumulation, not peptide-receptor contact. Even if Boltz-2 had returned a high ipTM, the biological interpretation of that result would require validation against this indirect-mechanism baseline.

WHAT THIS DOESN'T MEAN

DISCARDED is not disproved. This fold failed because the structural predictor could not converge on a stable docking pose — a tool-limit outcome that says nothing about whether the Aib-12 substitution actually rigidifies the YPR hinge, whether the RKLR patch aligns better as a helical face, or whether MOTS-c binds AMPK α2 at all. The indirect AICAR-mediated mechanism is the dominant published model, but the identification of direct MOTS-c protein-protein interactions (LARS1, PMID:39321430) establishes that direct binding mode is biologically plausible. No published study has directly measured MOTS-c–AMPK α2 binding affinity or excluded a direct interaction. The hypothesis that Pro-12 → Aib improves helical geometry and C-terminal face presentation remains chemically sound and experimentally untested.

WHAT WOULD ANSWER THE QUESTION

- **Biophysical binding assay (SPR or ITC):** Surface plasmon resonance or isothermal titration calorimetry with recombinant AMPK α2 catalytic domain would directly determine whether native MOTS-c and the Aib-12 analog bind the

catalytic subunit, and at what affinity — answering both the direct-binding question and the Aib modification question simultaneously.

- **Solution NMR / CD spectroscopy:** Circular dichroism or 2D NMR of native vs. Aib-12 MOTS-c in aqueous buffer (with and without TFE as a helix-promoting co-solvent) would directly measure whether Pro-12 → Aib rigidifies the C-terminal segment as hypothesized — the conformational premise of the fold.
- **Cellular AMPK activation assay with direct vs. indirect mechanism dissection:** Comparing MOTS-c and Aib-12 MOTS-c in a cell-free AMPK kinase assay (recombinant AMPK, no AICAR production pathway) vs. a cellular assay (with intact folate cycle) would distinguish direct from indirect mechanisms and whether the modification alters either pathway.
- **Free-energy perturbation (FEP) or enhanced sampling MD:** Classical or alchemical MD with an AMPK α2 structural template (PDB: 2Y94 or equivalent) would provide conformational and binding free-energy estimates for the Pro-12 → Aib substitution that are inaccessible to single-run AlphaFold-style predictors, especially for short peptides without template interfaces.

RAW METRICS

Metric	Value
pLDDT (peptide monomer)	0.619
pTM	0.550
ipTM	0.179
Chai-1 agreement	Not available
Boltz-2 affinity module	No values returned
Aggregation propensity (heuristic)	0.161 (low)
Stability score (heuristic)	0.319 (moderate)
BBB penetration (heuristic)	0.273 (low)
Half-life estimate (heuristic)	~30 min – 2 h (moderate)

All heuristic values are sequence-based estimates, not experimental measurements.

SEQUENCES

NATIVE

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MRWQEMGYIFYPRKLR
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MODIFIED

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MRWQEMGYIFY-Aib-RKLR
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CAVEATS

- in silico prediction only — requires wet lab validation
- single-run prediction (not ensembled)
- predicted properties may not reflect real-world biological behavior
- this is research, not medical advice
- ipTM 0.18 reflects predictor non-convergence at the interface, not a measured binding affinity — discard is a tool-limit outcome, not biological disproof
- the dominant literature mechanism for MOTS-c AMPK activation is indirect (AICAR-mediated), not direct peptide–AMPK α2 contact; the direct binding hypothesis is untested and requires biophysical validation
- Aib is a non-canonical amino acid not natively encoded; heuristic stability and half-life estimates do not account for potential immunogenicity or altered cellular uptake introduced by backbone methylation
- no Chai-1 cross-validation was available for this fold — single predictor result only
- heuristic peptide properties (aggregation, stability, BBB, half-life) are sequence-based estimates and should not be treated as experimental data

CITATIONS

1. **PMID —** (2015) — — The mitochondrial-derived peptide MOTS-c promotes metabolic homeostasis and reduces obesity and insulin resistance
2. **PMID —** (2023) — — MOTS-c Functionally Prevents Metabolic Disorders
3. **PMID —** (2023) — — MOTS-c: A promising mitochondrial-derived peptide for therapeutic exploitation
4. **PMID —** (2019) — — MOTS-c: A Mitochondrial-Encoded Regulator of the Nucleus
5. **PMID —** (2024) — — Mitochondrial-Derived Peptide MOTS-c Suppresses Ovarian Cancer Progression by Attenuating USP7-Mediated LARS1 Deubiquitination
6. **PMID —** (2022) — — The mitochondrial-derived peptide MOTS-c relieves hyperglycemia and insulin resistance in gestational diabetes mellitus
7. **PMID —** (2023) — — MOTS-c: A potential anti-pulmonary fibrosis factor derived by mitochondria
8. **PMID —** (2023) — — Role of MOTS-c in the regulation of bone metabolism
9. **PMID —** (2025) — — Redefining Mitochondrial Therapy for ME/CFS: The Case for MOTS-c

10. **PMID —** (2026) — — Humanin and MOTS-c Attenuate Atrial Fibrillation by Suppressing Fibrosis and Mitochondrial Dysfunction

SOLANA SIGNATURE HZHtfzEXHLaHyjQRqchj1GRcNoZHkZUm1MW5Bv3vmPvqcdHELsM1zo9A7Wf4NmCVoPdKD9XUd8h9dv19t61brue
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