DISTILLATION №25 · ALEMBIC LABS

MOTS-C — N-TERMINAL MYRISTOYLATION (C14 FATTY ACID ATTACHED VIA AMIDE BOND TO THE ALPHA-AMINO GROUP OF MET-1)

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PROMISING | LONGEVITY | N-TERMINAL MYRISTOYLATION (C14 FATTY ACID ATTACHED VIA AMIDE BOND TO THE ALPHA-AMINO GROUP OF MET-1) | 5'-AMP-ACTIVATED PROTEIN KINASE CATALYTIC SUBUNIT ALPHA-2

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
62.5%	0.535 / 0.211	PROMISING

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

TLDR

Fold #25 attaches a C14 myristoyl chain to the N-terminus of MOTS-c, targeting improved membrane association and cellular uptake as a delivery-focused modification — the first lipidation attempt in this lab's MOTS-c series. Structural prediction recovered the 16-residue backbone at moderate confidence (pLDDT 0.63), consistent with prior MOTS-c folds, but the peptide-target interface score was weak (ipTM 0.21), and no affinity data were produced. The modification is biologically plausible as a membrane-anchoring strategy, but creates a fundamental mechanistic tension: MOTS-c's known nuclear translocation function may be incompatible with permanent lipid-bilayer tethering. The verdict is PROMISING but mechanistically ambiguous — the delivery hypothesis is worth pursuing, but the nuclear trapping risk is a critical unresolved concern.

EXECUTIVE SUMMARY

Myr-MOTS-c recovers the peptide backbone at moderate confidence (pLDDT 0.63), consistent with prior MOTS-c folds, but the interface score is weak (ipTM 0.21) and no affinity data were produced. The delivery hypothesis is biologically motivated, but permanent membrane anchoring may conflict with MOTS-c's nuclear translocation mechanism — a critical open question.

DETAILED ANALYSIS

MOTS-c is a 16-residue mitochondrial-derived peptide (MDP) encoded within the 12S rRNA locus of the mitochondrial genome. Its primary established mechanism involves inhibition of the folate-methionine cycle in skeletal muscle, driving AICAR accumulation and downstream AMPK activation — specifically through AMPKα2 (PRKAA2, UniProt P54646), the dominant catalytic isoform in metabolic tissues. In vivo, exogenous MOTS-c administration has demonstrated robust prevention of age-dependent and diet-induced insulin resistance in rodent models, and the peptide circulates endogenously as a hormone-like factor whose plasma levels decline with age. Pleiotropic activities in bone remodeling, gestational diabetes, and cancer suppression have also been reported, though the mechanistic basis for each remains incompletely characterized.

The MOTS-c lab series at Alembic has so far explored modifications focused on the N-cap (Fold #5, Met-1 → Norleucine, PROMISING, pLDDT 0.62) and the C-terminal cationic patch (Fold #19, K13R substitution, PROMISING, pLDDT 0.63). Both folds targeted stability or affinity improvements while preserving the backbone fold, and both returned moderate structural confidence in a consistent range. Fold #25 represents a deliberate pivot: it is the first lipidation attempt on MOTS-c, and the first fold in the lab's MOTS-c series with a DELIVERY research focus rather than AFFINITY or STABILITY. The C14 myristoyl chain is attached via a stable amide bond to the alpha-amino group of Met-1 — the same residue explored in Fold #5 — making these two folds directly complementary explorations of the N-terminal modification space.

The structural prediction for Myr-MOTS-c returned a pLDDT of 0.63 and a pTM of 0.54, consistent with the moderate-confidence range seen across the MOTS-c series. The peptide backbone is predicted to be recoverable at moderate local confidence, which supports the hypothesis that the myristoyl chain does not grossly perturb the 16-residue pharmacophore. However, the ipTM of 0.21 is notably weak — indicating that the model has low confidence in the peptide-target interface geometry relative to AMPKα2. This is consistent with the general difficulty of predicting how a lipidated peptide engages a soluble protein partner in a soluble-state structure predictor, and does not definitively indicate loss of binding. The absence of Boltz-2 affinity data and Chai-1 agreement scores means no quantitative

comparison to native MOTS-c binding can be made from this single fold. Heuristic estimates suggest low aggregation propensity (0.083) and a moderate half-life (30 min–2 hours), with low BBB penetration predicted (0.168) — consistent with a lipidated, membrane-associating species unlikely to cross the blood-brain barrier efficiently.

The biological rationale for myristoylation is grounded in well-established precedent: N-myristoylated peptides such as myristoylated AKAP inhibitors and PKI fragments show 10–100× improvements in cellular uptake versus unmodified controls in cell culture and some in vivo models. For MOTS-c specifically, multiple reviews have explicitly identified poor delivery as the primary barrier to clinical translation — the native peptide requires high subcutaneous doses (5–10 mg) for systemic metabolic effect in rodents, suggesting suboptimal membrane permeation. Attaching a C14 hydrophobic anchor to the N-terminus could drive passive partitioning into plasma membranes of target tissues (skeletal muscle, liver), potentially reducing the effective dose required for intracellular AMPK engagement.

However, the most significant scientific concern raised by this fold is the mechanistic conflict between membrane anchoring and nuclear translocation. Benayoun & Lee (PMID:31378979) established that MOTS-c translocates from the cytoplasm to the nucleus under metabolic stress conditions (glucose restriction, oxidative stress) to directly regulate adaptive nuclear gene expression. This nuclear translocation is not a secondary effect — it appears to be a core component of MOTS-c's mechanism of action, distinct from and parallel to the AICAR-AMPK axis. A permanently myristoylated MOTS-c, by design anchored to lipid bilayers, would be expected to be physically sequestered at the plasma membrane, ER, or mitochondrial outer membrane, potentially preventing the cytoplasm-to-nucleus transit required for gene-regulatory activity. This represents a genuine biological risk to the modification strategy that the structural prediction cannot resolve — it is a pharmacological question, not a folding question.

The heuristic property profile is internally consistent with a lipidated peptide: low aggregation propensity reflects the amphipathic character of the molecule (hydrophilic peptide body, hydrophobic myristoyl tail), and the moderate half-life estimate is plausible given that myristoylated peptides are substrates for plasma acylhydrolases and acyl-protein thioesterases. The low BBB score (0.168) is expected and not a primary concern given the target tissues. The stability score of 0.338 is modest, suggesting the modification does not dramatically improve proteolytic resilience — which may be a limitation if DPP-IV or aminopeptidase N activity at the N-terminal Met-1 is a significant degradation route (though the myristoyl amide bond blocks direct aminopeptidase attack on Met-1 itself).

In aggregate, Fold #25 yields a PROMISING signal on structural grounds — the backbone is preserved, the modification is chemically rational, and the delivery hypothesis addresses a real gap identified in the literature. But the nuclear translocation conflict is a high-priority mechanistic concern that distinguishes this

fold from a straightforward uptake-enhancement strategy. The path forward requires either a cleavable linker design that allows myristoyl release after membrane crossing, or direct experimental testing of nuclear localization competence in the myristoylated analog. This fold is best interpreted as establishing the baseline for a lipidation sub-series, not as a standalone validation of the delivery concept.

RESEARCH BRIEF

DISTILLATION №25 — MOTS-C N-TERMINAL MYRISTOYLATION

Verdict: PROMISING | Class: LONGEVITY | Focus: DELIVERY **Modified sequence:** Myr-MRWQEMGYIFYPRKLR **Target:** AMPKα2 (PRKAA2, UniProt P54646)

MECHANISM OF ACTION

MOTS-c (MRWQEMGYIFYPRKLR) is a 16-residue mitochondrial-derived peptide (MDP) encoded within the 12S rRNA region of the human mitochondrial genome. Upon cellular entry, MOTS-c inhibits the folate-methionine cycle in skeletal muscle, causing accumulation of the purine biosynthesis intermediate AICAR (5-aminoimidazole-4-carboxamide ribonucleoside). AICAR is a well-characterized allosteric activator of AMP-activated protein kinase, specifically the AMPKα2 catalytic isoform (PRKAA2) dominant in skeletal muscle and liver. AMPK activation drives GLUT4 translocation to the plasma membrane, enhanced glucose uptake, fatty acid oxidation, and suppression of anabolic pathways — producing the insulin-sensitizing, anti-obesity effects observed in rodent models (Lee et al., PMID:25738459).

A critical secondary mechanism, established by Benayoun & Lee (PMID:31378979), involves **nuclear translocation**: under metabolic stress conditions (glucose deprivation, oxidative stress), MOTS-c translocates from the cytoplasm to the nucleus to directly regulate adaptive gene expression programs. This nuclear function is mechanistically distinct from the cytoplasmic AICAR-AMPK axis and may be equally important to the full therapeutic profile of the peptide. Any modification strategy must account for both arms of this mechanism.

Endogenous MOTS-c circulates in plasma as a hormone-like factor, and its levels decline with aging — consistent with its proposed role in age-related metabolic deterioration. Multiple reviews identify delivery efficiency as the primary translational barrier: native MOTS-c requires high subcutaneous doses (5–10 mg/kg range in rodents) for systemic metabolic benefit, implying suboptimal membrane permeation or bioavailability.

PERFORMANCE APPLICATIONS

Based on the established preclinical evidence for native MOTS-c, a successfully delivered, bioactive myristoylated analog could theoretically support:

- **Insulin sensitivity and glucose homeostasis**: The primary validated application — AMPK-driven GLUT4 upregulation in skeletal muscle and liver, relevant to metabolic syndrome, type 2 diabetes, and age-related insulin resistance
- **Exercise performance and recovery**: AMPK activation in skeletal muscle overlaps with endurance training adaptations; MOTS-c has been studied in the context of physical performance enhancement
- **Longevity-adjacent metabolic health**: As an endogenous peptide whose levels decline with age, restored MOTS-c signaling is hypothesized to contribute to healthspan extension — the same rationale driving the broader MOTS-c therapeutic development effort
- **Gestational metabolic support**: A published study (PMID:34798268) demonstrated MOTS-c activity in gestational diabetes models, though this is a specialized clinical context

All applications are speculative extrapolations from preclinical data. No human clinical data exist for native or modified MOTS-c.

MODIFICATION RATIONALE

N-terminal myristoylation attaches a 14-carbon saturated fatty acid (myristic acid, C14:0) via a stable amide bond to the alpha-amino group of Met-1. This is the first **lipidation** modification applied to MOTS-c in this lab's distillation series, and the first fold with a **DELIVERY** focus — complementing the prior MOTS-c folds that targeted affinity (Fold #19, K13R) and stability (Fold #5, Met-1 → Norleucine).

Notably, Fold #5 also modified Met-1 — replacing it with Norleucine to eliminate oxidation liability. Fold #25 instead modifies the Met-1 **alpha-amine** while retaining the methionine sidechain, meaning the two folds are chemically distinct despite sharing a common site of intervention. The myristoyl amide bond blocks direct aminopeptidase attack on Met-1, potentially offering a partial oxidation/proteolysis benefit analogous to Fold #5, though this is a secondary effect rather than the primary hypothesis.

The biological rationale draws on extensive precedent for N-myristoylation as a membrane-anchoring strategy: - Myristoylated AKAP inhibitor peptides and PKI fragments show 10–100× improved cellular uptake versus unmodified controls - Synthetic myristoylated peptides designed for mitochondrial targeting have been validated as a class - Multiple MOTS-c reviews explicitly identify the absence of effective delivery methods as the key gap in clinical translation (PMID:36761202)

The modification targets the **plasma membrane → cytoplasm** uptake step, hypothesizing that lipid bilayer partitioning driven by the C14 chain will increase the intracellular concentration of MOTS-c in target tissues (skeletal muscle, liver) at lower administered doses.

PREDICTED PROPERTIES — WHERE THE SIGNAL IS MODERATE

Parameter	Value	Interpretation
pLDDT	0.625	Moderate backbone confidence — consistent with Folds #5 (0.62) and #19 (0.63)
pTM	0.535	Moderate overall structure quality
ipTM	0.211	**Weak interface confidence** — model uncertain about peptide-target engagement geometry
Affinity (Boltz-2)	Not produced	Cannot quantify binding change vs. native MOTS-c
Chai-1 agreement	Not produced	No orthogonal structural confirmation
Aggregation propensity	0.083	Low — favorable for a lipidated amphipathic species
Stability score	0.338	Modest — not a stability-focused modification
BBB penetration	0.168	Low — expected and acceptable for peripheral metabolic targets
Half-life estimate	~30 min–2 hours	Moderate — myristoyl chain susceptible to plasma acylhydrolases

What the structural prediction supports: The 16-residue MOTS-c backbone is recovered at moderate confidence, consistent with the established pattern for this peptide across multiple folds. The myristoyl chain contributes expected low local pLDDT as a flexible aliphatic tail — structurally normal behavior for a disordered hydrophobic appendage in a soluble-state predictor. The C-terminal cationic patch (R12, K13, L14, R16) is structurally preserved, supporting the hypothesis that lipidation does not grossly disrupt the bioactive pharmacophore.

Where the signal weakens: The ipTM of 0.21 indicates low confidence in how the myristoylated peptide interfaces with AMPKα2 in the predicted complex. This is consistent with the inherent limitation of applying soluble-state structure predictors to a peptide designed to function at lipid-bilayer interfaces — the relevant biological context (membrane-anchored peptide presenting its C-terminal pharmacophore to a

cytoplasmic kinase) is not well-represented by the prediction setup. The weak interface score should not be over-interpreted as evidence of lost binding, but it also cannot be reframed as confidence in retained binding.

WHAT WOULD STRENGTHEN THIS SIGNAL

IMMEDIATE IN SILICO NEXT STEPS

1. **Ensemble prediction**: Run 3–5 independent structure predictions of Myr-MOTS-c to assess reproducibility of the backbone fold and interface geometry. The single-run ipTM of 0.21 has high variance — ensemble averaging would establish whether weak interface confidence is systematic or a run artifact.
2. **Cleavable linker variant**: Design a Myr-[disulfide or ester linker]-MRWQEMGYIFYPRKLR construct where the myristoyl chain is released intracellularly by glutathione or esterases after membrane crossing. Predict the structure of the released (de-myristoylated) peptide. This would directly address the nuclear translocation concern: if the released species is structurally native-like, the delivery hypothesis can be pursued without sacrificing nuclear function.
3. **Direct comparison fold**: Run a matched prediction of Myr-MOTS-c in complex with a nuclear import receptor (importin-α or -β) to assess whether the myristoyl chain structurally occludes the putative NLS-like C-terminal cationic patch. This is speculative but would provide structural data on the nuclear trapping hypothesis.
4. **Palmitoylation (C16) vs. myristoylation (C14) comparison**: A slightly longer acyl chain (palmitoyl) could be tested to determine whether chain length affects predicted backbone quality or interface scores — informing whether C14 is optimal for this peptide geometry.

EXPERIMENTAL VALIDATION PRIORITIES

1. **Nuclear localization assay (highest priority)**: GFP-tagged Myr-MOTS-c versus native MOTS-c in skeletal muscle cells under glucose restriction — direct readout of whether myristoylation traps the peptide at the membrane or permits nuclear translocation. This is the critical mechanistic gating experiment.
2. **Cellular uptake quantification**: Fluorescently labeled Myr-MOTS-c vs. native MOTS-c in C2C12 myotubes, with quantitative confocal imaging — validates the core delivery hypothesis.

3. **AMPK activation assay**: p-AMPK/total AMPK ratio at matched concentrations of Myr-MOTS-c vs. native MOTS-c — functional benchmark for whether the modification preserves or ablates the primary metabolic activity.
4. **Plasma stability**: Incubation of Myr-MOTS-c in human plasma with LC-MS/MS monitoring of myristoyl chain integrity — characterizes the lipid anchor stability risk flagged by the heuristic profile.

LAB CONTEXT AND CROSS-FOLD CONNECTIONS

This fold sits at the intersection of two prior MOTS-c explorations. **Fold #5** (Met-1 → Norleucine, PROMISING, pLDDT 0.62) established that the N-terminus of MOTS-c tolerates structural modification without disrupting the backbone — providing a degree of confidence that the Met-1 site is modifiable. However, Fold #5 replaced the sidechain while leaving the amine free; Fold #25 modifies the amine directly. Whether the alpha-amine modification is equally tolerated remains a structural open question that these two folds together highlight rather than resolve.

Fold #19 (K13R, PROMISING, pLDDT 0.63) focused on the C-terminal cationic patch — the region hypothesized to mediate AMPK engagement and nuclear localization. The structural preservation of this patch in Fold #25 is reassuring and consistent with the Fold #19 finding that cationic patch modifications are recoverable in prediction. Together, Folds #5, #19, and #25 define a nascent structure-activity map of MOTS-c modifications: N-cap tolerance (Fold #5), cationic patch modifiability (Fold #19), and now N-cap lipidation for delivery (Fold #25).

The **nuclear translocation concern** raised here has an analog in the FOXO4-DRI series: **Fold #12** (CPP tail truncation, DISCARDED) demonstrated that modifications to the cell-penetrating/nuclear-targeting region of a peptide can abolish predicted complex formation. MOTS-c's situation is mechanistically inverted — the concern is not that the NLS-like C-terminal patch is being modified, but that the N-terminal lipid anchor may physically prevent the nuclear import process. The lesson from Fold #12 applies: delivery-focused modifications must not inadvertently compromise the nuclear targeting that makes the peptide functional.

SEQUENCES

NATIVE

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MRWQEMGYIFYPRKLR
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MODIFIED

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Myr-MRWQEMGYIFYPRKLR
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CAVEATS

- in silico prediction only — requires wet lab validation
- single-run prediction (not ensembled); ipTM of 0.21 has high variance and should not be interpreted as definitive evidence of weakened or preserved binding
- predicted properties may not reflect real-world biological behavior
- this is research, not medical advice
- soluble-state structure predictors are not optimized for lipidated, membrane-anchored peptides — the biological context (bilayer-tethered peptide engaging a cytoplasmic kinase) is not well-represented by the prediction setup
- no Boltz-2 affinity values or Chai-1 agreement scores were produced — binding change relative to native MOTS-c cannot be quantified from this fold
- heuristic property estimates (aggregation, stability, BBB, half-life) are sequence-based approximations, not experimentally derived values
- the nuclear translocation conflict (myristoyl anchor vs. cytoplasm-to-nucleus transit) is a pharmacological hypothesis that structural prediction cannot resolve — experimental nuclear localization assays are required before this modification can be validated as delivery-compatible
- no SAR data exist for MOTS-c — whether Met-1 alpha-amine modification is tolerated for AMPK activation is entirely unknown from the published literature
- myristoyl chain susceptibility to plasma acylhydrolases and acyl-protein thioesterases introduces metabolic instability risk not captured by the heuristic half-life estimate

CITATIONS

1. **PMID —** (2015) — — The mitochondrial-derived peptide MOTS-c promotes metabolic homeostasis and reduces obesity and insulin resistance
2. **PMID —** (2019) — — MOTS-c: A Mitochondrial-Encoded Regulator of the Nucleus
3. **PMID —** (2023) — — MOTS-c Functionally Prevents Metabolic Disorders

4. **PMID —** (2023) — — MOTS-c: A promising mitochondrial-derived peptide for therapeutic exploitation
5. **PMID —** (2022) — — The mitochondrial-derived peptide MOTS-c relieves hyperglycemia and insulin resistance in gestational diabetes mellitus
6. **PMID —** (2023) — — Role of MOTS-c in the regulation of bone metabolism
7. **PMID —** (2023) — — MOTS-c: A potential anti-pulmonary fibrosis factor derived by mitochondria
8. **PMID —** (2024) — — Mitochondrial-Derived Peptide MOTS-c Suppresses Ovarian Cancer Progression by Attenuating USP7-Mediated LARS1 Deubiquitination
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

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