MOTS-C — MET-1 → NORLEUCINE (NLE) SUBSTITUTION; ISOSTERIC REPLACEMENT OF THE OXIDATION-PRONE N-TERMINAL METHIONINE WITH A NON-OXIDIZABLE STRAIGHT-CHAIN ANALOG

generated 2026-05-02T12:52:47.979416+00:00

PROMISING LONGEVITY

MET-1 → NORLEUCINE (NLE) SUBSTITUTION; ISOSTERIC REPLACEMENT OF THE OXIDATION-PRONE N-TERMINAL METHIONINE WITH A NON-OXIDIZABLE STRAIGHT-CHAIN ANALOG

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

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
61.6%	0.574 / 0.418	PROMISING

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

TLDR

MOTS-c is a 16-amino acid mitochondrial-derived peptide that activates AMPK through folate cycle disruption, with established roles in metabolic homeostasis and longevity signaling. This fold explores replacing the oxidation-prone N-terminal methionine (Met-1) with norleucine (Nle), a classical isosteric, non-oxidizable methionine mimetic. Structure prediction returns a pLDDT of 0.62 and ipTM of 0.42 — moderate confidence consistent with a short, partially ordered peptide — and heuristic profiling suggests the substitution is structurally silent without introducing steric penalties. The signal is promising rather than conclusive: the chemical rationale is sound and precedented, but the absence of any MOTS-c SAR literature or resolved binding interface means validation remains entirely ahead of us.

EXECUTIVE SUMMARY

MOTS-c Met-1→Nle: pLDDT 0.62, ipTM 0.42 — moderate confidence, no steric red flags. Isosteric substitution predicted to eliminate N-terminal oxidation liability. Strong chemical precedent; no MOTS-c SAR data exists to confirm. Promising, not yet refined.

DETAILED ANALYSIS

MOTS-c is a 16-amino acid peptide encoded within the 12S rRNA locus of mitochondrial DNA, first characterized in 2015 as a regulator of insulin sensitivity and skeletal muscle metabolism. Its canonical mechanism involves inhibition of the intracellular folate-methionine cycle, causing accumulation of AICAR — a naturally occurring AMPK activator — which in turn engages the AMPK catalytic alpha-2 subunit (PRKAA2) to drive downstream metabolic reprogramming. Beyond this pathway, MOTS-c is now understood to be a pleiotropic mitonuclear signaling molecule: under metabolic stress it translocates to the nucleus and directly modulates adaptive gene expression, influencing GLUT4, NRF2, STAT3, and IL-10 among others. Circulating MOTS-c levels decline with age, and exogenous peptide administration has shown efficacy in preclinical models of diet-induced obesity, insulin resistance, gestational diabetes, and even ovarian cancer suppression via LARS1 interaction. It sits squarely within the longevity peptide class.

The modification under investigation in this distillation is a single-residue substitution at position 1: methionine is replaced by norleucine (Nle), a non-proteinogenic amino acid carrying a straight four-carbon alkyl side chain in place of methionine's thioether. The oxidation hypothesis is chemically rigorous — N-terminal methionines are among the most oxidation-vulnerable residues in any peptide, readily forming methionine sulfoxide under physiological oxidative conditions or during formulation storage. Norleucine has been widely deployed as a pharmaceutical stabilization strategy in peptide drug development, including in GLP-1 and oxytocin analogs, precisely because it is isosteric and isosteric with methionine in terms of van der Waals volume and hydrophobicity, while being chemically inert to oxidation. The rationale for applying it to MOTS-c is therefore well-grounded in precedent.

Structure prediction was performed using AlphaFold-class modeling targeting the AMPK alpha-2 catalytic subunit (PRKAA2). The predicted complex returned a pLDDT of 0.616 and a pTM of 0.574, with an interface-specific ipTM of 0.418. These values are characteristic of short peptides (16 residues) engaging a large protein partner — some disorder at termini is expected and does not by itself indicate a failed prediction. The caption from the structural agent describes the Nle-1 side chain occupying a hydrophobic envelope geometrically consistent with Met-1, with no steric clashes arising from the sulfur-to-methylene swap. This is exactly what an

isosteric substitution should produce, and the model is consistent with the 'structurally silent' hypothesis.

Heuristic sequence-based profiling adds complementary signal. The aggregation propensity score is low (0.083), which is favorable for a peptide therapeutic — this modification does not increase aggregation risk. The stability score is moderate (0.542), and the estimated half-life sits in the 30-minute to 2-hour range, which for a peptide of this class is a reasonable baseline that the Nle substitution is predicted to improve over the oxidation-labile native sequence. BBB penetration is low (0.149), consistent with MOTS-c's primarily peripheral and intracellular (skeletal muscle, liver) sites of action rather than any central nervous system target.

From the literature, a critical uncertainty shadows all structural interpretations: no co-crystal structure or cryo-EM structure of MOTS-c bound to any target exists. The AMPK pathway engagement appears to be mediated through upstream metabolic intermediates (AICAR) rather than direct AMPK binding, and MOTS-c's interactions with LARS1 and nuclear targets are protein-protein interactions whose structural determinants are unresolved. This means neither the importance of Met-1 to any binding interface nor its dispensability has been empirically tested. The 'N-cap geometry' rationale, while chemically intuitive, is inferential rather than evidence-based. There is also a secondary concern: Met-1 in a mitochondrially-encoded peptide may be a recognition element for methionine aminopeptidase or N-terminal acetyltransferase processing — substitution with Nle would block such post-translational events, potentially altering intracellular trafficking or stability through a different mechanism than oxidation.

What elevates this fold to PROMISING rather than DISCARDED is the combination of: (1) strong chemical precedent for Nle as a Met replacement in therapeutic peptides, (2) a structurally reasonable prediction with no red flags such as steric clashes or dramatic backbone deviation, (3) a genuine and unaddressed oxidative stability liability in the native sequence, and (4) a biologically important target with preclinical proof-of-concept. What prevents a REFINED verdict is the absence of Chai-1 independent confirmation, the low ipTM (0.42) reflecting real uncertainty at the interface level, and the complete lack of MOTS-c-specific SAR data to anchor any confidence in functional tolerance of this substitution.

This distillation establishes a chemically motivated, structurally plausible baseline for MOTS-c oxidative stabilization. The most valuable next experiments would be parallel synthesis of native and Nle-1 MOTS-c with direct oxidative stress challenge assays, followed by cell-based AMPK activation readouts (phospho-ACC, phospho-AMPK) to confirm the modification is pharmacologically neutral. As the lab accumulates folds across the MOTS-c series, this Nle-1 variant provides a clean reference point for future modifications targeting other residues — particularly the internal Met-6 (position 6, sequence MRWQEMG…), which represents a second oxidation liability not addressed here.

RESEARCH BRIEF

FOLD №16 — MOTS-C MET1→NLE SUBSTITUTION

Verdict: PROMISING | Class: Longevity | Target: AMPK α2 (PRKAA2)

MECHANISM OF ACTION

MOTS-c is a 16-amino acid mitochondrial-derived peptide (MDP) encoded within the 12S rRNA locus of mitochondrial DNA. Its primary mechanism operates intracellularly: MOTS-c inhibits the folate-methionine cycle in skeletal muscle and other metabolic tissues, causing accumulation of AICAR (5-aminoimidazole-4-carboxamide ribonucleotide) — a naturally occurring AMP-mimetic. AICAR in turn activates 5'-AMP-activated protein kinase, specifically the catalytic alpha-2 subunit (PRKAA2/AMPK-α2), triggering downstream glucose uptake, fatty acid oxidation, and mitochondrial biogenesis. Beyond this canonical pathway, MOTS-c translocates to the nucleus under metabolic stress, directly modulating adaptive gene expression including NRF2, GLUT4, STAT3, and IL-10 — consistent with its identity as a pleiotropic mitonuclear communication molecule. More recently, interaction with LARS1 (leucyl-tRNA synthetase) has been reported in oncological contexts, expanding its known protein-protein interaction landscape. Circulating MOTS-c declines with age, and exogenous administration is bioactive in preclinical models of obesity, insulin resistance, and metabolic aging.

PERFORMANCE APPLICATIONS

MOTS-c has demonstrated preclinical efficacy across several contexts directly relevant to metabolic performance and longevity:

- **Insulin sensitivity & glucose metabolism**: AMPK-α2 activation improves skeletal muscle glucose uptake and GLUT4 translocation, with demonstrated reversal of age-dependent and diet-induced insulin resistance in rodent models.
- **Obesity resistance**: Exogenous MOTS-c reduces adiposity and improves lipid metabolism in diet-induced obesity models.
- **Metabolic aging**: Declining MOTS-c levels are associated with the metabolic phenotype of aging; restoration of circulating levels is a proposed longevity intervention rationale.
- **Gestational metabolic health**: Efficacy in gestational diabetes models extends potential applications.

- **Proposed emerging contexts**: ME/CFS (AMPK/NRF2 rationale, preprint-stage evidence) and atrial fibrillation (alongside humanin, preprint-stage). Both remain speculative and low-evidence at this time.

MODIFICATION RATIONALE

The native MOTS-c sequence (MRWQEMGYIFYPRKLR) opens with methionine at position 1. N-terminal methionines are among the most oxidation-vulnerable residues in peptide chemistry: they are solvent-exposed, subject to reactive oxygen species in biological fluids, and readily oxidized to methionine sulfoxide — a modification that can reduce peptide potency, increase proteolytic susceptibility, and limit shelf-life in formulation.

Norleucine (Nle) is the classical pharmaceutical solution. It carries a straight four-carbon alkyl side chain (n-butyl) in place of methionine's thioether, producing: - **Near-identical van der Waals volume and hydrophobicity** — the substitution is isosteric in all practically relevant senses. - **No oxidizable sulfur atom** — complete elimination of the Met oxidation liability. - **Backbone geometry unchanged** — Nle is an alpha-amino acid with standard L-configuration and no unusual conformational preferences.

This substitution strategy is well-precedented in therapeutic peptide development (GLP-1 analogs, oxytocin analogs, and others). Application to MOTS-c is novel, and the field contains no prior SAR data for any MOTS-c analog — this fold represents the first in silico exploration of this modification.

A secondary consideration: Met-1 in mitochondrially-encoded peptides may serve as a substrate for methionine aminopeptidase (MAP) or N-terminal acetyltransferase (NAT) processing. Nle substitution would block any such co-translational or post-translational N-terminal modification. Whether this affects the biology of exogenously administered synthetic MOTS-c is unknown, but it warrants awareness in downstream interpretation.

PREDICTED PROPERTIES — WHERE SIGNAL IS MODERATE

Parameter	Value	Interpretation
pLDDT	0.616	Moderate — typical for short peptide, not a failure signal
pTM	0.574	Moderate global fold confidence
ipTM	0.418	

Parameter	Value	Interpretation
		Low-moderate interface confidence — real uncertainty at binding surface
Chai-1 agreement	Not available	Single-model prediction — not independently confirmed
Aggregation propensity	0.083	Low — favorable; Nle does not increase aggregation risk
Stability score	0.542	Moderate — consistent with heuristic improvement over native Met
BBB penetration	0.149	Low — expected; MOTS-c's targets are peripheral/ intracellular
Half-life estimate	~30 min – 2 hr	Moderate; Nle substitution predicted to reduce oxidative degradation

Structural note: The predicted structure shows the Nle-1 side chain occupying a hydrophobic envelope geometrically consistent with native Met-1, with no steric clashes introduced by the sulfur→methylene swap. The backbone topology is consistent with a short, partially ordered peptide. This is exactly the profile expected of a structurally silent isosteric substitution — but at ipTM 0.42, the interface prediction should not be over-interpreted.

What the heuristics suggest, not guarantee: modest improvement in oxidative half-life, no degradation of aggregation behavior, preservation of the hydrophobic N-terminal character. All heuristic estimates only — these are sequence-derived proxies, not wet-lab measurements.

WHAT WOULD STRENGTHEN THIS SIGNAL

Additional in silico work: - **Ensemble prediction**: Run 5+ AlphaFold-Multimer seeds and report median ipTM with variance — a single-run ipTM of 0.42 is insufficient to characterize interface confidence. - **Chai-1 independent confirmation**: The absence of Chai-1 agreement data leaves this as a single-model prediction; Chai-1 consensus would significantly strengthen the structural verdict. - **Boltz-2 affinity module**: Predicted ΔΔG or binding affinity change would directly test the 'pharmacologically neutral' hypothesis. - **Native MOTS-c fold reference**: Running the unmodified sequence (MRWQEMGYIFYPRKLR) through the same pipeline would enable direct RMSD comparison of backbone and side-chain geometry at position 1, providing the 'structurally silent' confirmation currently inferred rather than demonstrated. - **Met-6 variant**: The internal Met at position 6 (MRWQE**M**GYIFYPRKLR) is a second oxidation liability not addressed by this fold. A

double-substitution Nle-1/Nle-6 variant would offer more comprehensive oxidative protection and is a logical next candidate in this series.

Wet-lab experiments that would be definitive: - **Parallel synthesis + oxidative challenge**: Synthesize native MOTS-c and (Nle-1)MOTS-c; expose both to H_2O_2 or accelerated oxidative stress conditions; quantify methionine sulfoxide formation by LC-MS. This directly tests the stability hypothesis. - **Cell-based AMPK activation assay**: Treat myotubes (e.g., C2C12) with equimolar native vs. Nle-1 MOTS-c; measure phospho-AMPK-α2 (Thr172) and phospho-ACC by Western blot. This tests pharmacological neutrality of the substitution. - **Cellular uptake comparison**: Fluorescently label both variants and compare uptake kinetics in skeletal muscle cells to detect any N-terminus-dependent membrane interaction differences. - **Plasma stability assay**: Incubate both variants in human plasma and measure intact peptide by LC-MS/MS over 0–4 hours to quantify in vitro half-life improvement.

This is the first MOTS-c fold in the Alembic Labs series. Future folds exploring Met-6, C-terminal modifications, or cyclic variants will be cross-referenced against this Nle-1 baseline.

SEQUENCES

NATIVE

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MRWQEMGYIFYPRKLR
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MODIFIED

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(Nle)RWQEMGYIFYPRKLR
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CAVEATS

- In silico prediction only — requires wet-lab synthesis and biological validation before any conclusions can be drawn about real-world activity or stability.
- Single-run prediction (not ensembled) — ipTM 0.42 is based on one model seed; ensemble prediction across multiple seeds is needed to characterize confidence variance.
- Predicted properties may not reflect real-world biological behavior — heuristic scores (aggregation propensity, stability, half-life, BBB penetration) are sequence-derived proxies, not measured values.
- This is research, not medical advice — MOTS-c has no approved clinical indications; all described applications are preclinical or investigational.

- No MOTS-c SAR data exists in the literature — functional tolerance of any substitution at any position, including Met-1, is entirely uncharacterized empirically.
- No resolved structure of MOTS-c bound to any target — the 'structurally silent at the binding interface' conclusion is inferred from isosteric chemistry, not confirmed by a co-crystal or cryo-EM reference structure.
- Nle substitution blocks potential N-terminal methionine processing (MAP/NAT) — biological consequences of this for exogenous synthetic peptide are unknown.
- Chai-1 independent structural confirmation was not available for this fold — the structural verdict rests on a single prediction platform.
- The oxidation liability of native MOTS-c has not been empirically quantified in the literature — the magnitude of the stability problem being solved is assumed by analogy, not demonstrated for this specific peptide.

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 —** (2022) — — The mitochondrial-derived peptide MOTS-c relieves hyperglycemia and insulin resistance in gestational diabetes mellitus
6. **PMID —** (2024) — — Mitochondrial-Derived Peptide MOTS-c Suppresses Ovarian Cancer Progression by Attenuating USP7-Mediated LARS1 Deubiquitination
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 5k2kreEFCTkEyb1aeVVcAGVz61P5k8UmbBJsNQETgMVPm77Tp8t5ExzyzLPCFBE343Eb8LwDauxENGxwx3vQxjuw
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