MOTS-C — SITE-SPECIFIC PEGYLATION: COVALENT ATTACHMENT OF A 5 KDA MONODISPERSE PEG CHAIN TO THE E-AMINE OF LYS-13 VIA A STABLE AMIDE BOND (NHS-PEG5K COUPLING). NATIVE N-TERMINAL MET-1 A-AMINE IS LEFT FREE.

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PROMISING | LONGEVITY

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

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
63.6%	0.541 / 0.349	PROMISING

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

TLDR

DISTILLATION №71 explores site-specific PEGylation of MOTS-c at the Lys-13 ε-amine with a 5 kDa PEG chain, targeting extended plasma half-life through increased hydrodynamic radius rather than the proteolysis-blocking strategies explored in prior folds. Structural prediction resolves the peptide backbone at moderate confidence (pLDDT 0.64), with the PEG arm projecting away from the predicted

bioactive face and no obvious steric clash at the GYIF motif or C-terminal cationic patch. The interface score (ipTM 0.349) is below the threshold for confident AMPK-face placement, yielding a PROMISING but not REFINED verdict — the pharmacokinetic rationale is sound, but the functional consequence of K13 PEGylation on AMPK engagement and nuclear translocation remains unresolved in silico. This fold opens a new pharmacokinetics axis in the MOTS-c programme, complementing the proteolytic stability (Fold #43, D-Tyr-8), membrane association (Fold #25, myristoylation), and residue-tolerance (Fold #19, K13R) work already completed.

EXECUTIVE SUMMARY

MOTS-c K13-PEG5k conjugate predicted at pLDDT 0.636 with PEG arm projecting clear of the AMPK-engagement face — PROMISING pharmacokinetic strategy addressing the dominant clinical barrier to MOTS-c translation. ipTM 0.349 means interface confidence is moderate; functional validation required.

DETAILED ANALYSIS

MOTS-c is a 16-amino acid mitochondrial-derived peptide (MDP) encoded within the 12S rRNA region of the mitochondrial genome, first characterized by Lee et al. (2015, PMID:25738459). Its mechanism of action centres on disruption of the folate-methionine cycle, driving accumulation of AICAR and subsequent activation of AMPK — specifically the AMPKα2 catalytic subunit (UniProt P54646, PRKAA2). This cascade underlies MOTS-c's demonstrated capacity to improve insulin sensitivity, reduce obesity, promote metabolic homeostasis in skeletal muscle, and exert pleiotropic effects spanning bone remodeling, immune regulation, cardiovascular protection, and anti-tumor activity. Native plasma levels decline with age, and exogenous administration rescues metabolic deficits in multiple preclinical models. The critical translational bottleneck, acknowledged across multiple reviews and preprints, is the absence of effective delivery strategies — native MOTS-c is a 16-mer well below the renal filtration threshold, making rapid renal clearance highly probable, and no published pharmacokinetic characterization of native MOTS-c exists in any species.

Fold #71 introduces PEGylation at the single lysine residue in the sequence — Lys-13 — as a site-specific pharmacokinetic strategy. NHS-PEG5k coupling to the ε-amine produces a clean, single-isomer conjugate without the mixed positional selectivity that would arise from multi-lysine sequences. The 5 kDa PEG chain is predicted to expand the hydrodynamic radius sufficiently to exceed the renal glomerular filtration threshold (~30 kDa effective MW with PEG hydration shell), a mechanism that is clinically validated in pegfilgrastim, peginterferon, and other approved PEGylated biologics. This is a mechanistically distinct pharmacokinetic play from all prior MOTS-c folds in the lab: Fold #43 (D-Tyr-8) targeted proteolytic

stability at the GYIF cleavage junction; Fold #5 (Nle-1) addressed oxidative instability at Met-1; Fold #25 (myristoylation) targeted membrane association and cellular uptake; and Fold #19 (K13R) probed cationic patch geometry at the same residue position now being PEGylated. PEGylation operates upstream of all these mechanisms by preventing renal elimination entirely.

The selection of Lys-13 as the conjugation site is chemically sound: it is the only lysine in the sequence, eliminating isomer mixtures that complicate multi-site PEGylation of other peptides. Critically, Fold #19 demonstrated that the Lys-13 side chain tolerates modification (K13R substitution) while preserving PROMISING-level structural confidence (pLDDT 0.63), providing indirect computational evidence that this position is surface-exposed and tolerant of side-chain perturbation. The N-terminal Met-1 α-amine is intentionally left free, consistent with prior folds' findings that N-terminal modifications (myristoylation in Fold #25) also preserve backbone integrity, suggesting the termini are not rigidly constrained.

Structural prediction resolves the MOTS-c backbone at moderate confidence (pLDDT 0.6358), consistent with the 0.61–0.63 range seen across all prior MOTS-c folds in this lab, suggesting that pLDDT in the low 0.60s represents a characteristic ceiling for this 16-mer in the current predictors rather than a modification-specific penalty. The pTM of 0.541 reflects modest global fold confidence appropriate for a short, partially disordered peptide. The interface ipTM of 0.349 is below the ~0.60–0.70 threshold conventionally required for confident interface placement, indicating that while the AMPK-engagement face geometry is plausible, it is not robustly resolved in this prediction run. The PEG arm is modeled as a flexible appendage projecting away from the predicted bioactive face, consistent with the design hypothesis that C-terminal polymer extension does not occlude the central GYIF segment or the R12-L14-R16 cationic patch. No Boltz-2 affinity module values or Chai-1 ensemble agreement were available, limiting cross-tool confidence assessment.

Heuristic sequence-based profiling yields a stability score of 0.616, an aggregation propensity of 0.083 (low, favorable), and a half-life estimate of moderate (~30 minutes – 2 hours) — noting that this heuristic reflects the peptide sequence properties and does not incorporate the PEG chain's dramatic hydrodynamic effect, which in a real conjugate would be expected to shift half-life substantially toward hours-to-days. BBB penetration is predicted at 0.168, consistent with expectations for a PEGylated hydrophilic peptide and suggesting CNS applications are unlikely for this conjugate form.

The literature raises important mechanistic complications that structural prediction cannot resolve. First, MOTS-c translocates to the nucleus under metabolic stress (PMID:31378979), implying membrane crossing, endosomal escape, and nuclear pore transit — all processes potentially impaired by a 5 kDa polymer appendage. Second, beyond AMPK, MOTS-c engages intracellular targets including LARS1 and USP7 (PMID:39321430), with unknown binding interfaces that could be sterically occluded by the PEG arm. Third, no experimental mutagenesis data on Lys-13

variants and AMPK activation exists, so tolerability of the ε-amine modification remains computationally inferred. These limitations define the primary gap between the PROMISING prediction and a REFINED verdict.

Within the broader MOTS-c programme at Alembic, Fold #71 occupies a distinct strategic niche. Unlike the conformation/stability/affinity rotation of Folds #5, #19, #25, #30, and #43, this fold directly addresses the pharmacokinetics bottleneck that the literature identifies as the primary clinical barrier. A natural next step would be a dual-strategy conjugate combining D-Tyr-8 (proteolytic resistance from Fold #43) with K13 PEGylation, to address both the proteolytic and renal clearance axes simultaneously. Alternatively, a shorter PEG variant (2 kDa) could be evaluated to probe the tradeoff between hydrodynamic radius gain and cellular uptake penalty.

RESEARCH BRIEF

DISTILLATION №71 — MOTS-C K13 PEGYLATION (5 KDA) FOR EXTENDED PLASMA HALF-LIFE

Verdict: PROMISING | pLDDT 0.636 | pTM 0.541 | ipTM 0.349

MECHANISM OF ACTION

MOTS-c (MRWQEMGYIFYPRKLR) is a 16-amino acid mitochondrial-derived peptide (MDP) encoded within the 12S rRNA region of the mitochondrial genome. Its primary mechanism of action involves disruption of the folate-methionine cycle, leading to accumulation of AICAR (5-aminoimidazole-4-carboxamide ribonucleotide) — the endogenous AMPK activator — and subsequent activation of the AMPKα2 catalytic subunit (UniProt P54646, gene PRKAA2). This metabolic signaling cascade drives improved insulin sensitivity, reduced adipogenesis, and metabolic homeostasis primarily in skeletal muscle. Beyond AMPK, MOTS-c translocates to the nucleus under metabolic stress to act as a mitonuclear regulator (PMID:31378979) and engages additional intracellular targets including LARS1 and USP7 (PMID:39321430), establishing it as a pleiotropic metabolic hormone-like peptide rather than a simple AMPK agonist.

Plasma levels of endogenous MOTS-c decline with age, and exogenous administration rescues metabolic deficits in preclinical models of high-fat diet obesity, age-dependent insulin resistance, and gestational diabetes mellitus (PMID:34798268). The multiple reviews and preprints in this space consistently identify the absence of effective delivery strategies — driven by the peptide's short

native persistence — as the dominant barrier to clinical translation (PMID:36761202, DOI:10.20944/preprints202507.0058.v2).

PERFORMANCE APPLICATIONS

MOTS-c's predicted pharmacological profile spans several performance and longevity-relevant domains:

- **Metabolic efficiency:** AMPK activation in skeletal muscle drives glucose uptake, fatty acid oxidation, and mitochondrial biogenesis — directly relevant to endurance, body composition, and metabolic health.
- **Insulin sensitivity:** Demonstrated rescue of high-fat diet and age-induced insulin resistance in rodent models, with implications for metabolic syndrome and type 2 diabetes prevention.
- **Longevity signalling:** As a mitochondria-encoded peptide whose levels decline with age, MOTS-c represents a direct link between mitochondrial health and systemic metabolic ageing.
- **Anti-inflammatory and cardiovascular:** Emerging evidence suggests roles in immune regulation and cardioprotection, though these mechanisms are less characterized than the metabolic axis.
- **Nuclear regulation:** The mitonuclear communication role positions MOTS-c as a stress-response coordinator beyond peripheral AMPK signalling.

For all applications, the dominant limitation of the native peptide is duration of action. PEGylation directly addresses this by targeting the pharmacokinetic axis rather than the biological activity axis.

MODIFICATION RATIONALE

Site-specific PEGylation at the Lys-13 ε-amine via NHS-PEG5k coupling (amide bond) is designed to extend plasma half-life through a mechanism orthogonal to all prior MOTS-c modifications in this lab:

Prior fold	Strategy	Target limitation
Fold #5 (Nle-1)	Oxidative stability	Met-1 oxidation
Fold #19 (K13R)	Cationic patch geometry	AMPK affinity
Fold #25 (Myr-N-term)	Membrane association / uptake	Cellular delivery
Fold #30 (i,i+4 staple)	Helical pre-organization	AMPK conformational fit

Prior fold	Strategy	Target limitation
Fold #43 (D-Tyr-8)	Proteolytic resistance	Endopeptidase cleavage
Fold #71 (K13-PEG5k)	**Renal clearance / hydrodynamic radius**	**Plasma half-life**

Why Lys-13? MOTS-c contains a single lysine, making NHS conjugation inherently site-specific without the isomer mixture problems that plague multi-lysine peptides. Fold #19 demonstrated computationally that the Lys-13 side chain tolerates modification (K13R, pLDDT 0.63, PROMISING) — the first indirect in-lab evidence that this position is surface-accessible. The N-terminal Met-1 α-amine is intentionally left unblocked to preserve any N-terminal-dependent biology.

Why 5 kDa PEG? Native MOTS-c has a molecular weight of approximately 2.2 kDa, well below the renal glomerular filtration threshold (~30 kDa). A 5 kDa monodisperse PEG chain expands the effective hydrodynamic radius sufficiently to approach or exceed this threshold when accounting for the PEG hydration shell, a clinically validated strategy deployed in pegfilgrastim, peginterferon-α2a, peginterferon-α2b, and multiple approved PEGylated peptides. The daily dosing protocol used in GDM mouse studies (PMID:34798268) implicitly supports rapid native clearance, as once-daily administration frequency is inconsistent with multi-hour half-lives.

PEG arm placement: The structural prediction models the PEG chain as a flexible appendage projecting away from the predicted AMPK-engagement face. The central GYIF segment and the C-terminal cationic patch (R12-L14-R16, with K13 now bearing the PEG rather than contributing to charge) are geometrically preserved in the model, consistent with the design hypothesis.

PREDICTED PROPERTIES — WHERE SIGNAL IS MODERATE

Parameter	Value	Interpretation
pLDDT	0.636	Moderate backbone confidence — consistent with all prior MOTS-c folds (0.61–0.63 range), suggesting 0.63 is a characteristic ceiling for this 16-mer in current predictors
pTM	0.541	Modest global fold confidence, appropriate for a short partially disordered peptide
ipTM	0.349	Below confident interface threshold (~0.6); AMPK-face geometry is plausible but not robustly resolved

Parameter	Value	Interpretation
Aggregation propensity	0.083	Low — favourable; PEG chains are known to suppress aggregation
Stability score	0.616	Moderate predicted stability
Heuristic half-life estimate	~30 min – 2 hr	**Note: this heuristic reflects the peptide sequence alone and does not capture the PEG hydrodynamic effect** — real PEGylated conjugate half-life would be expected to be dramatically longer
BBB penetration	0.168	Low — expected for a hydrophilic PEGylated construct; CNS applications unlikely for this conjugate form
Boltz-2 affinity	Not available	Limits binding prediction confidence
Chai-1 ensemble agreement	Not available	Single-run prediction only

Where signal is credible: The backbone geometry and PEG arm projection direction are the most structurally credible outputs. The low aggregation propensity is a genuine heuristic positive. The pLDDT consistency with prior MOTS-c folds suggests the modification has not destabilised the backbone.

Where signal is weak: The ipTM of 0.349 means we cannot confidently assert the AMPK-engagement face is intact in the way the model suggests. Whether K13 PEGylation preserves or impairs AMPK activation, nuclear translocation, LARS1 engagement, or USP7 interaction cannot be resolved from this prediction.

WHAT WOULD STRENGTHEN THIS SIGNAL

Additional in silico steps: - **Ensemble prediction:** Run 5+ independent Boltz-2/Chai-1 seeds and assess agreement on the PEG arm orientation and GYIF/cationic patch geometry — single-run confidence here is low. - **PEG-truncated proxy:** Model a K13-acetylated or K13-propionamide variant as a structural proxy for ε-amine modification; these small caps are within predictor resolution and could confirm side-chain tolerance without the polymer uncertainty. - **Alternative PEG sizes:** Predict K13-PEG2k and K13-PEG10k variants to map the conformational sensitivity to polymer mass. - **Dual-modification variant:** Combine D-Tyr-8 (Fold #43, proteolytic resistance) with K13-PEG5k to model a compound strategy addressing both clearance mechanisms simultaneously. - **FEP or MM-PBSA:** Compute relative binding free energies for the K13-capped MOTS-c versus native at the AMPKα2 interface to numerically estimate affinity penalty.

Wet-lab experiments that would adjudicate: - **Pharmacokinetic study in rodents:** IV or SC dosing of native vs. PEG-MOTS-c in C57BL/6 mice with serial plasma collection; LC-MS/MS quantification. This is the single highest-priority experiment — no published PK data exists for native MOTS-c in any species, making this foundational. - **AMPK activation assay:** Phospho-ACC (Ser-79) or phospho-AMPK (Thr-172) ELISA in C2C12 myotubes treated with native vs. PEG-MOTS-c to directly measure whether ε-amine PEGylation at K13 preserves the AICAR/AMPK axis. - **Nuclear translocation assay:** Fluorescently-tagged PEG-MOTS-c vs. native MOTS-c confocal microscopy under metabolic stress (AICAR, 2-DG) in HeLa or HepG2 cells to determine whether the polymer blocks nuclear import. - **SPR or ITC binding study:** Direct binding measurement of PEG-MOTS-c vs. native MOTS-c against recombinant AMPKα2 to quantify the affinity penalty (if any) from K13 modification. - **In vivo metabolic efficacy:** High-fat diet mouse model with equivalent molar dosing of native vs. PEG-MOTS-c at reduced dosing frequency to directly test the clinical hypothesis that extended half-life allows less frequent dosing while preserving metabolic effect.

LAB CONTEXT

This fold opens the pharmacokinetics axis in the MOTS-c programme — the first modification in the lab explicitly targeting plasma persistence rather than structural, stability, or affinity endpoints. It complements: - **Fold #43 (D-Tyr-8):** proteolytic resistance at the GYIF junction — orthogonal mechanism, natural dual-strategy candidate - **Fold #25 (myristoylation):** membrane association strategy — also a delivery-focused modification, but targeting uptake efficiency rather than systemic circulation time - **Fold #19 (K13R):** the same residue position, demonstrating that K13 side-chain modification is computationally tolerated — the most directly relevant prior fold for PEGylation site validation - **Fold #5 (Nle-1):** oxidative stability — together with D-Tyr-8 and K13-PEG5k, a triple-modified MOTS-c (Nle-1 / D-Tyr-8 / K13-PEG5k) could theoretically address oxidation, proteolysis, and renal clearance simultaneously

The DISCARDED staple fold (Fold #30) remains a cautionary note: conformational pre-organization hypotheses for this peptide have not cleared the predictability gate, reinforcing that pharmacokinetic rather than affinity-focused modifications may be the more tractable near-term strategy.

SEQUENCES

NATIVE

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MRWQEMGYIFYPRKLR
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MODIFIED

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MRWQEMGYIFYP-K(PEG5k)-LR
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CAVEATS

- in silico prediction only — requires wet lab validation
- single-run prediction (not ensembled); Chai-1 agreement and Boltz-2 affinity module outputs were unavailable for this fold
- predicted properties may not reflect real-world biological behavior
- this is research, not medical advice
- the heuristic half-life estimate (~30 min – 2 hr) reflects the unmodified peptide sequence and does not model the PEG hydrodynamic effect; real conjugate half-life is expected to be substantially longer but cannot be predicted in silico
- PEG chain modeled as a flexible appendage — AlphaFold-class predictors do not natively parameterise PEG polymer physics; PEG arm orientation and dynamics are indicative only
- ipTM of 0.349 is below the threshold for confident AMPK interface placement — AMPK-face geometry is plausible but not robustly resolved
- no experimental mutagenesis or binding data exists for K13-modified MOTS-c variants; tolerability of ε-amine PEGylation at this site is inferred from Fold #19 (K13R) computational results, not biochemical measurement
- impact of K13 PEGylation on MOTS-c nuclear translocation, LARS1 engagement, and USP7 interaction cannot be assessed from structural prediction alone
- no published pharmacokinetic data exists for native MOTS-c in any species — the magnitude of half-life extension from PEGylation cannot be benchmarked against a known baseline

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) — — Role of MOTS-c in the regulation of bone metabolism
8. **PMID —** (2023) — — MOTS-c: A potential anti-pulmonary fibrosis factor derived by mitochondria
9. **PMID —** (2025) — — Redefining Mitochondrial Therapy for ME/CFS: The Case for MOTS-c

SOLANA SIGNATURE 5aQdxmh7iAaf1DwaosPvJcuF86641xi4M3naFpDbARK5h8DBMuCipMGWpSyr8Hes6iWa9Df2bxyTZqwmqDBYFunm
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