MOTS-C — LYS-13 → ARG SUBSTITUTION (K13R), CONVERTING THE CENTRAL KLR MOTIF INTO AN RLR MOTIF TO CREATE A CONTIGUOUS ARG-RICH CATIONIC PATCH WITH THE C-TERMINAL ARG-16

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PROMISING | LONGEVITY

LYS-13 → ARG SUBSTITUTION (K13R), CONVERTING THE CENTRAL KLR MOTIF INTO AN RLR MOTIF TO CREATE A CONTIGUOUS ARG-RICH CATIONIC PATCH WITH THE C-TERMINAL ARG-16

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

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
62.7%	0.541 / 0.499	PROMISING
TARGET	**UNIPROT**	**BINDING PROBABILITY**
5'-AMP-activated protein kinase catalytic subunit alpha-2	P54646	—

TLDR

DISTILLATION №19 explores a Lys-13 → Arg substitution on MOTS-c, converting the C-terminal KLR motif into RLR to create a more uniform guanidinium-based cationic patch hypothesized to enhance engagement with AMPK's regulatory surface. AlphaFold returned pLDDT 0.627 and ipTM ~0.499 — structurally coherent but short of high-confidence binding, earning a PROMISING verdict. The principal caveat is mechanistic: the dominant literature model positions MOTS-c as an indirect AMPK activator via AICAR accumulation rather than a direct AMPK-binding ligand, meaning the cationic patch optimization may modulate other biological interactions (cell

penetration, LARS1-like direct contacts) more than AMPK activation per se. The fold produces a useful structural hypothesis and a rational analog for downstream wet-lab SAR work, but it cannot resolve the indirect-vs-direct mechanism question in silico.

EXECUTIVE SUMMARY

MOTS-c K13R (MRWQEMGYIFYPRRLR): pLDDT 0.627, ipTM 0.499 — structurally coherent, consistent with Fold #5's conservative-substitution pattern. Promising cationic patch design, but MOTS-c's AMPK activation is predominantly indirect via AICAR; direct binding evidence needed to confirm the electrostatic engagement hypothesis.

DETAILED ANALYSIS

MOTS-c is a 16-residue mitochondrial-derived peptide (MDP) encoded within the mitochondrial 12S rRNA locus, first characterized by Lee et al. (2015) as a regulator of insulin sensitivity and metabolic homeostasis. Its canonical mechanism operates through disruption of the folate-methionine cycle, causing intracellular AICAR accumulation and consequent allosteric AMPK activation — placing AMPK alpha-2 (PRKAA2) at the center of virtually every described MOTS-c bioactivity, from skeletal muscle glucose uptake to anti-aging gene expression programs. The peptide also translocates to the nucleus under metabolic stress and has been shown to engage protein targets directly, most notably LARS1 in an ovarian cancer context, demonstrating that its surface chemistry is functionally relevant beyond metabolic metabolite modulation.

The native MOTS-c sequence (MRWQEMGYIFYPRKLR) presents a structurally interesting C-terminal cationic cluster: Arg-12, Lys-13, Leu-14, and Arg-16 together create a positively charged tail that is solvent-exposed and likely responsible for membrane and protein-surface engagement. DISTILLATION №19 converts Lys-13 to Arg, yielding MRWQEMGYIFYPRRLE — wait, correctly: MRWQEMGYIFYPRRLR — a single-residue substitution that homogenizes the cationic chemistry from a mixed Arg/Lys tail to a pure Arg tail. The design rationale is grounded in well-established biophysical principles: arginine's planar guanidinium group can form bidentate salt bridges and cation-π interactions that are geometrically constrained, pH-stable across a wider range than lysine's ammonium, and typically longer-lived at acidic protein surfaces. If MOTS-c does contact an acidic regulatory surface on AMPK alpha-2, the K13R variant is predicted to engage it more persistently.

Structural prediction (AlphaFold, single run) returned pLDDT 0.627 — essentially identical to the Fold #5 Nle-1 variant (pLDDT 0.62), which is expected given that both substitutions are highly conservative and neither touches the hydrophobic core (residues 4-11, WQEMGYIFY). The pTM of 0.541 and ipTM of 0.499 describe a

complex model where the overall peptide fold is moderate-confidence but the specific binding interface sits at the boundary of interpretability. The structural caption notes that the Arg-13 guanidinium is oriented toward solvent/target in a manner consistent with the design hypothesis. No Chai-1 corroboration or Boltz-2 affinity output was available, meaning this is a single-predictor, single-run pose — a hypothesis-consistent model, not a validated complex.

Heuristic sequence-based properties suggest a manageable profile: aggregation propensity 0.083 (low — the added Arg residue does not meaningfully increase aggregation risk for this 16-mer), stability score 0.452 (moderate), blood-brain barrier penetration 0.224 (low, as expected for a cationic peptide), and estimated half-life in the moderate range (~30 min–2 hours). The K→R swap is unlikely to significantly alter proteolytic susceptibility at this position, though the increased guanidinium density could marginally affect membrane partitioning and intracellular trafficking compared to the native sequence.

The most important intellectual tension in this fold is mechanistic rather than structural. The literature consensus positions MOTS-c as an indirect AMPK activator — it disrupts folate cycle metabolism, generating AICAR, which then allosterically activates AMPK. No published study has characterized direct MOTS-c–AMPK physical interaction, nor mapped an acidic surface on AMPK alpha-2 that is engaged by the peptide's cationic tail. This means the hypothesis of 'enhanced AMPK pathway activation via stabilized cationic patch' rests on an unconfirmed premise: that MOTS-c contacts AMPK directly at all. The K13R substitution may well prove biologically interesting — it could enhance membrane penetration, modulate LARS1 or other direct-contact targets, or alter nuclear translocation efficiency — but AMPK activation improvement specifically through direct electrostatic engagement remains speculative until a direct interaction is confirmed.

Cross-fold context is instructive here. Fold #5 established that conservative single-residue substitutions on MOTS-c yield structurally coherent predictions at pLDDT ~0.62 without disrupting the predicted backbone — DISTILLATION №19 recapitulates that pattern precisely, reinforcing confidence in the structural prediction while inheriting the same mechanistic uncertainties about what the C-terminal cationic tail actually does. The contrast with Fold #6 (Epitalon D-Ala, pLDDT 0.34, DISCARDED) and the FOXO4-DRI truncation (Fold #12, DISCARDED) highlights that MOTS-c tolerates conservative substitutions well in silico, making it a tractable scaffold for iterative analog design even if the precise mechanism of improvement requires biological validation.

In summary, DISTILLATION №19 yields a structurally credible MOTS-c analog with a rationally strengthened cationic C-terminus, predicted to fold comparably to the native peptide while presenting a geometrically superior electrostatic surface. The PROMISING verdict reflects genuine structural signal constrained by deep mechanistic uncertainty. The fold most usefully serves as a candidate for direct experimental comparison against native MOTS-c in AMPK activation assays — if

K13R outperforms native despite the indirect mechanism, it would suggest cell-surface or intracellular trafficking improvements are also at play. If it does not, it would provide the first published SAR data point for MOTS-c residue contribution, which would itself be a meaningful contribution to an essentially unmapped structure-activity landscape.

RESEARCH BRIEF

DISTILLATION №19 — MOTS-C K13R (MRWQEMGYIFYPRRLR)

Verdict: PROMISING | pLDDT 0.627 | ipTM 0.499 | Class: LONGEVITY

MECHANISM OF ACTION

MOTS-c is a 16-residue mitochondrial-derived peptide (MDP) encoded within the mitochondrial genome's 12S rRNA locus. Its primary described mechanism is metabolic rather than receptor-mediated: MOTS-c disrupts the intracellular folate-methionine cycle, causing accumulation of AICAR (5-aminoimidazole-4-carboxamide ribonucleotide), an endogenous allosteric activator of AMPK (5'-AMP-activated protein kinase). AMPK activation downstream of this cascade drives the full spectrum of MOTS-c's described bioactivities — improved glucose uptake in skeletal muscle, attenuation of insulin resistance, anti-obesity effects, bone metabolism regulation, and anti-inflammatory signaling.

Critically for this fold, the literature also documents that MOTS-c can engage protein targets through direct binding: its interaction with LARS1 (leucyl-tRNA synthetase 1) in an ovarian cancer context demonstrates that the peptide's surface chemistry participates in specific protein–protein contacts, not only metabolic perturbation. Under metabolic stress, MOTS-c also translocates to the nucleus and regulates adaptive gene expression directly. This dual — indirect metabolic and direct protein-binding — mode of action is the conceptual foundation for hypothesizing that optimizing MOTS-c's surface electrostatics could enhance target engagement potency.

PERFORMANCE APPLICATIONS

The MOTS-c K13R variant, if validated, would target the same therapeutic and performance contexts as native MOTS-c:

- **Metabolic health & insulin sensitivity**: AMPK activation in skeletal muscle is the primary mechanism; improved engagement could translate to more potent glucose disposal and insulin sensitization
- **Longevity / metabolic aging**: MOTS-c's AMPK-activating role connects directly to longevity pathways (FOXO signaling, mitophagy, mitochondrial biogenesis)
- **Gestational diabetes and reproductive metabolic disorders**: MOTS-c efficacy in gestational diabetes models (PMID:34798268) is AMPK-dependent; a more potent analog would be directly relevant
- **Oncology support**: Direct protein-binding capability (LARS1) suggests K13R may modulate cancer-relevant interactions; cationic patch optimization could alter selectivity
- **Pulmonary and cardiac applications**: MOTS-c studies in pulmonary fibrosis (PMID:37307934) and atrial fibrillation contexts depend on AMPK and mitochondrial protection pathways

Performance biohacking applications center on metabolic optimization, exercise recovery (AMPK-driven adaptations), and potential healthy aging support — consistent with the LONGEVITY classification.

MODIFICATION RATIONALE

Native MOTS-c (MRWQEMGYIFYPR**K**LR) carries a mixed cationic C-terminal cluster: Arg-12, **Lys-13**, Leu-14, Arg-16. The K13R substitution (→ MRWQEMGYIFYPRRRLR... correctly: MRWQEMGYIFYPRRRLR — specifically MRWQEMGYIFYPR**R**LR) converts this mixed tail into a uniform Arg-Leu-Arg arrangement, homogenizing the positive charge chemistry without altering residue count, charge state, or hydrophobic core composition.

The biophysical rationale is well-grounded: - **Guanidinium vs. ammonium**: Arg's guanidinium group (pKa ~12.5) remains protonated at physiological pH more reliably than Lys's ammonium (pKa ~10.5), providing more consistent positive charge in biological microenvironments - **Bidentate geometry**: Guanidinium forms two simultaneous hydrogen bonds with carboxylate groups (bidentate salt bridges), geometrically constraining the contact and increasing dwell time at acidic protein surfaces - **Cation-π capacity**: Arg participates in cation-π interactions with aromatic protein residues; Lys does so less effectively - **No structural disruption**: K→R is among the most conservative natural substitutions — both are long, positively charged, flexible side chains with no core packing role at position 13

This parallels the conservative-substitution philosophy of **Fold #5** (Met-1 → Nle), which applied an isosteric swap at the N-terminus to prevent oxidation without disrupting backbone geometry, yielding pLDDT 0.62 — essentially matched by this fold at 0.627. The design explicitly avoids the N-terminal D-amino acid failure mode seen in **Fold #6** (Epitalon, pLDDT 0.34), applying instead a physiochemically homologous substitution at a solvent-exposed C-terminal residue.

PREDICTED PROPERTIES (WHERE SIGNAL IS MODERATE)

Property	Native MOTS-c (Fold #5 reference)	K13R Variant (Fold #19)	Signal Confidence
pLDDT	~0.62	0.627	Moderate — consistent
pTM	~0.54	0.541	Moderate
ipTM (binding interface)	—	0.499	Moderate — boundary of interpretability
Aggregation propensity	—	0.083 (low)	Low-risk
Stability score	—	0.452 (moderate)	Moderate
BBB penetration	—	0.224 (low)	Expected for cationic peptide
Half-life estimate	—	~30 min – 2 hr	Moderate — similar to native
Chai-1 agreement	—	Not available	⚠ Single-predictor only
Boltz-2 affinity	—	Not available	⚠ No quantitative ΔΔG

Key observations: - The structural prediction is **internally consistent with the conservative-substitution hypothesis**: pLDDT 0.627 falls in the same moderate-confidence band as Fold #5, suggesting the backbone fold is not disrupted by K13R - The ipTM of 0.499 represents a plausible but not definitive binding interface — the model generates a hypothesis-consistent complex geometry but cannot confirm it - Low aggregation propensity (0.083) is favorable: despite increased Arg density, the 16-mer does not appear to gain aggregation-prone character, supporting solution behavior comparable to the native peptide - The moderate stability score (0.452) and half-life estimate are unremarkable — no predicted stability gain from K13R

alone, consistent with the substitution being charge-homogenizing rather than structure-hardening

The **moderate signal** here reflects a structurally credible prediction constrained by: (a) absence of multi-predictor consensus, (b) absence of affinity quantification, and (c) the fundamental uncertainty about whether MOTS-c engages AMPK alpha-2 directly at all.

WHAT WOULD STRENGTHEN THIS SIGNAL

Computational next steps: 1. **Chai-1 ensemble prediction** of the K13R variant vs. native MOTS-c docked to AMPK alpha-2 — multi-predictor consensus would substantially increase confidence in the binding pose and allow direct structural comparison 2. **Boltz-2 affinity module** (when available for peptide-protein complexes) to generate a predicted ΔΔG for K13R vs. native, providing quantitative binding change hypothesis 3. **Molecular dynamics (MD) simulation** of the C-terminal cationic tail in the predicted complex — would reveal whether the Arg-13 guanidinium forms stable bidentate contacts or remains freely diffusing, directly testing the core hypothesis 4. **Native MOTS-c baseline fold** at identical conditions to directly compare pLDDT/ipTM metrics rather than relying on Fold #5 as a proxy (Fold #5 was N-terminal; the C-terminal environment may differ) 5. **K13A (alanine scan)** fold — a predicted null substitution that would establish whether K13 contributes to structural confidence at all, providing an in silico negative control

Experimental validation path: 1. **AMPK alpha-2 activation assay** (cell-free or cellular): Direct comparison of native MOTS-c vs. K13R in AICAR-independent conditions would test whether any component of MOTS-c's AMPK activation is direct and Arg-13-dependent 2. **SPR or BLI binding kinetics** against recombinant AMPK alpha-2: Would resolve the fundamental question of whether MOTS-c contacts AMPK directly and whether K13R improves kd (off-rate) 3. **Cellular AMPK phosphorylation assay** (pAMPK T172): Direct comparison in C2C12 myotubes or HEK293 cells expressing PRKAA2, controlling for AICAR-mediated indirect effects where possible 4. **Protease stability panel**: K→R is not expected to alter tryptic susceptibility (both are trypsin cleavage sites), but the relative stability of K13R vs. native in plasma should be confirmed experimentally 5. **LARS1 binding comparison**: Given MOTS-c's established direct interaction with LARS1, testing K13R vs. native in the LARS1 context would probe whether cationic patch optimization alters direct protein-binding selectivity and potency

> **Critical mechanistic experiment**: If MOTS-c's AMPK activation is entirely indirect (folate cycle → AICAR → AMPK), then K13R may show equivalent AMPK activation to native in cellular assays but potentially differ in cell-penetrating efficiency, nuclear translocation rate, or LARS1-like direct interactions. Distinguishing these pathways experimentally

would determine whether the K13R modification is functionally meaningful and through which mechanism.

SEQUENCES

NATIVE

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MRWQEMGYIFYPRKLR
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MODIFIED

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MRWQEMGYIFYPRRLR
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CAVEATS

- in silico prediction only — requires wet lab validation
- single-run prediction (not ensembled) — Chai-1 corroboration unavailable for this fold
- predicted properties may not reflect real-world biological behavior
- this is research, not medical advice
- the dominant literature mechanism for MOTS-c AMPK activation is indirect (folate cycle → AICAR), not direct peptide–AMPK binding; the cationic patch optimization hypothesis assumes a direct interaction that has not been experimentally confirmed
- no published SAR data exist for MOTS-c — contribution of Lys-13 to bioactivity is experimentally unknown
- heuristic peptide properties (aggregation, stability, BBB, half-life) are sequence-based estimates, not wet-lab measurements
- ipTM 0.499 sits at the boundary of confident binding interface prediction — the complex geometry is hypothesis-consistent, not validated
- no Boltz-2 affinity output available — quantitative predicted binding change (ΔΔG) cannot be reported for this fold
- K→R substitution retains trypsin cleavage susceptibility at position 13; proteolytic stability improvement is not predicted from this modification alone

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

SOLANA SIGNATURE 2MrQDrfk58Bvvs2kQvgApje4hCRicttrrHggXhw3EoF2pAJhh18oK7rSFi7xWVzdGqoYcgtqGTrU7wmaTZS63UJZ
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