HUMANIN — N-TERMINAL MYRISTOYLATION: COVALENT ATTACHMENT OF MYRISTIC ACID (C14 SATURATED FATTY ACID) TO THE A-AMINE OF MET-1 VIA AMIDE BOND, YIELDING MYR-MAPRGFSCLLLLTSEIDLPVKRRA

generated 2026-05-04T08:52:35.318584+00:00

FAILED LONGEVITY N-TERMINAL MYRISTOYLATION: COVALENT ATTACHMENT OF MYRISTIC ACID (C14 SATURATED FATTY ACID) TO THE A-AMINE OF MET-1 VIA AMIDE BOND, YIELDING MYR-MAPRGFSCLLLLTSEIDLPVKRRA APOPTOSIS REGULATOR BAX

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
—	— / —	FAILED
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Apoptosis regulator BAX	Q07812	—

TLDR

Fold #59 attempted to predict the structure of N-terminal myristoylated Humanin (Myr-MAPRGFSCLLLLTSEIDLPVKRRA) in complex with the apoptosis regulator BAX, targeting mitochondrial outer membrane enrichment as a mechanism to enhance BAX antagonism. The prediction failed at the technical level: Boltz-2 exited without producing a PDB file, and Chai-1 cross-validation was not run, leaving the complex entirely unevaluated by structural tools. Heuristic sequence-based profiling confirms the native Humanin hydrophobic hotspot at residues 7–20 but provides no information about lipid placement or BAX engagement. This is a tool-limit failure, not a biological verdict on the myristoylation strategy.

EXECUTIVE SUMMARY

Fold #59 — Humanin myristoylation for MOM-targeted BAX antagonism — hit a hard tool limit: Boltz-2 produced no structure for the lipidated peptide. No structural verdict possible; wet-lab synthesis and ITC/SPR remain the only path forward.

DETAILED ANALYSIS

Humanin (HN) is a 24-amino acid mitochondria-derived peptide (MDP) with one of the most thoroughly validated anti-apoptotic mechanisms in the longevity peptide space. Its core biology — direct binding to BAX, prevention of BAX conformational activation, and suppression of mitochondrial outer membrane permeabilization (MOMP) — is established across biochemical, cellular, and biophysical studies going back to Guo et al. 2003. The 2019 Morris et al. work added a structural dimension, showing HN and BAX co-assemble into fibers in solution, with BAX's membrane-targeting C-terminal helix required for the interaction. This body of literature makes BAX an unusually well-supported target for a longevity peptide, and HN one of the more mechanistically credible peptides in this lab's portfolio.

The hypothesis tested in Fold #59 was membrane targeting by lipidation: attaching myristic acid (C14 saturated fatty acid) to the α-amine of Met-1 via an amide bond to create Myr-MAPRGFSCLLLLTSEIDLPVKRRA. The rationale draws on the well-established role of myristoylation in directing proteins to membranes — Src-family kinases, BID/tBID, MARCKS — and the fact that BAX insertion and oligomerization is a mitochondrial outer membrane (MOM) event. If BAX inhibition is intrinsically membrane-proximate (as Morris et al. 2019 imply), then co-localizing HN to the MOM via a myristoyl anchor could increase effective local concentration and enhance BAX engagement kinetics.

However, this hypothesis carries meaningful biological tension that the literature surfaces. Guo et al. 2003 showed that HN prevents BAX translocation to the MOM — meaning the key inhibitory event likely occurs in the cytosol, before membrane insertion. Locking HN to the MOM via myristoylation could theoretically cause it to arrive 'too late' in the BAX activation sequence. Morris et al.'s in vitro fiber data, generated without membranes, further suggests cytosolic HN-BAX engagement is real and substantial. Additionally, myristoylation alone is frequently insufficient for selective MOM targeting without a complementary electrostatic 'second signal', raising the risk of non-specific membrane partitioning across ER, plasma membrane, and nuclear envelope. The loss of BimEL antagonism (a cytosolic function of native HN, per Luciano et al. 2005) is an additional concern if membrane anchoring sequesters the peptide away from cytosolic BH3-only proteins.

Despite these biological nuances, the fold did not reach a structural verdict — the failure is purely technical. Boltz-2 exited without producing a PDB file. The most likely cause is the non-standard chemical entity: myristoylation is a covalent lipid

modification (myristic acid amide-bonded to the N-terminal amine) that falls outside the canonical amino acid alphabet and requires either explicit small-molecule parameterization or a CCD ligand definition that current AlphaFold-derived pipelines do not natively handle. This is the same class of tool-limit failure seen in Fold #44 (palmitoyl-γGlu-Lys lipidation on Epitalon, pLDDT 0.34, DISCARDED) and Fold #58 (PEG2-TAT chimera on Epitalon, FAILED), both involving non-standard chemical conjugates that stress current prediction infrastructure.

Heuristic sequence-based analysis of the unmodified Humanin sequence confirms the expected hydrophobic character: aggregation propensity 0.31 with a hotspot at residues 7–20 consistent with the known amphipathic helix, stability score 0.37, and moderate-to-long predicted half-life (1–6 hours). These metrics describe the native peptide core only and say nothing about the myristoyl tail's membrane insertion geometry, its effect on BAX groove access, or whether the lipid sterically clashes with the α1-α2 region of BAX. No pLDDT, pTM, or ipTM values were generated.

In the context of this lab's Humanin series, Fold #59 is the third distinct modification strategy tested on this peptide. Fold #22 introduced a disulfide bridge (Cys-8/S14C, PROMISING, pLDDT 0.56) to pre-organize the central helix for BAX groove engagement — a structure-stabilization approach. Fold #37 tested S7A substitution to bias activity toward intracellular BAX inhibition (DISCARDED, pLDDT 0.62) — a receptor-selectivity approach. Fold #59 now tests lipid-mediated membrane delivery — a pharmacokinetic/localization approach. The three folds together map out the major strategic axes for HN optimization: structural pre-organization, target selectivity, and membrane targeting. The first two produced usable structural data (even if modest); this third produced none.

The failure here is a tool gap, not a scientific dead end. Myristoylated peptides are experimentally accessible — solid-phase synthesis with a myristoyl NHS ester or myristoyl-CoA enzymatic transfer are both routine — and the BAX interaction is measurable by ITC, SPR, or the liposome permeabilization assay formats used in the original Humanin literature. The membrane-targeting hypothesis is biologically coherent even if the structural data to evaluate it remains absent. Fold #59 should be understood as a staging ground for wet-lab work rather than a failed prediction.

RESEARCH BRIEF

DISTILLATION №59 — FAILED

HUMANIN N-TERMINAL MYRISTOYLATION FOR MITOCHONDRIAL MEMBRANE TARGETING / BAX ANTAGONISM

TLDR

Fold #59 was **DISCARDED due to a tool-limit failure**: Boltz-2 exited without producing a PDB file when presented with N-terminal myristoylated Humanin (Myr-MAPRGFSCLLLLTSEIDLPVKRRA) in complex with BAX. This is a **non-standard chemical entity failure** — the myristoyl amide bond to Met-1 falls outside the canonical amino acid alphabet that current AlphaFold-derived pipelines handle natively. No structural verdict on the hypothesis was reached. This is not a biological invalidation.

WHAT WE TRIED

Humanin (HN, MAPRGFSCLLLLTSEIDLPVKRRA) is a 24-amino acid mitochondria-derived peptide whose direct binding to BAX — preventing its conformational activation and translocation to the mitochondrial outer membrane (MOM) — is one of the most mechanistically supported anti-apoptotic interactions in the longevity peptide literature (Guo et al. 2003, Morris et al. 2019).

The hypothesis in Fold #59 was that covalent attachment of myristic acid (C14 saturated fatty acid) to the α-amine of Met-1 via an amide bond would enrich HN at the MOM — the precise site where BAX inserts and oligomerizes during apoptosis induction. The rationale draws on the validated membrane-targeting function of myristoylation in Src-family kinases, BID/tBID, and MARCKS, and on the Morris et al. 2019 finding that BAX's membrane-targeting C-terminal helix is required for HN-induced fiber formation, implying the HN–BAX interaction is intrinsically membrane-proximate. The expectation was that Boltz-2 would model the Humanin helix (residues 5–19) docking into the BAX hydrophobic groove with the myristoyl chain projecting toward a hydrophobic surface patch, yielding pLDDT >0.65 in the helical core.

This fold is the third in the lab's Humanin series, following Fold #22 (disulfide cyclization Cys-8/S14C → PROMISING, pLDDT 0.56) and Fold #37 (S7A substitution for receptor selectivity → DISCARDED, pLDDT 0.62). Those two folds addressed structural pre-organization and target selectivity, respectively. Fold #59 introduced a third strategic axis: **pharmacokinetic membrane delivery**.

WHY IT WAS DISCARDED

Boltz-2 exited without generating a PDB file. Chai-1 cross-validation was not run. The primary cause is the **non-standard chemical entity**: N-terminal myristoylation is a covalent lipid modification that requires explicit small-molecule parameterization (e.g., a CCD ligand code, SMILES input, or modified residue definition) that current AlphaFold-derived folding pipelines do not handle within the

standard amino acid alphabet. The myristoyl amide at Met-1 cannot be represented as a sequence character, and without a coordinate template or ligand definition, the predictor cannot place or score it.

This is the same class of failure seen in **Fold #44** (palmitoyl-γGlu-Lys lipidation on Epitalon, pLDDT 0.34, DISCARDED) and **Fold #58** (PEG2-TAT chimera on Epitalon, FAILED) — both involving non-peptidic conjugates that exceed current tool resolution. The pattern is consistent: lipid-modified and PEGylated peptides are a systematic blind spot for this generation of structure predictors.

No structural metrics were produced. Heuristic sequence analysis of the unmodified Humanin backbone yielded: aggregation propensity 0.31 (hotspot residues 7–20, consistent with the known amphipathic helix), stability score 0.37, BBB penetration 0.16, and a moderate-to-long estimated half-life (1–6 hours). These describe the native peptide core only and carry no information about myristoyl tail geometry, BAX groove compatibility, or lipid-mediated membrane partitioning.

WHAT THIS DOESN'T MEAN

FAILED is not "disproved." The myristoylation-mediated membrane targeting hypothesis for Humanin has not been evaluated — it has been rendered invisible by current tool limitations. The biological logic remains coherent: BAX engagement is a membrane-proximate event, myristoylation is a validated MOM-targeting strategy, and co-localizing HN with BAX at the site of insertion is a mechanistically grounded approach. There is also genuine biological tension in the hypothesis — Guo et al. 2003 showed HN prevents BAX translocation to the MOM, implying cytosolic inhibition is the primary mechanism, and membrane anchoring could theoretically arrive too late in the activation sequence. Morris et al.'s in vitro fiber data (generated without membranes) further supports cytosolic HN-BAX engagement as real and substantial. These are scientific questions worth adjudicating experimentally, not computational artifacts. The absence of a structural prediction says nothing about whether myristoyl-HN would bind BAX, partition to the MOM, or enhance anti-apoptotic activity in cells.

WHAT WOULD ANSWER THE QUESTION

- **Solid-phase synthesis + ITC or SPR**: Myristoyl-HN is synthetically accessible via N-terminal myristoyl NHS ester coupling. Isothermal titration calorimetry or surface plasmon resonance against recombinant BAX (with and without lipid vesicles to mimic MOM context) would directly measure binding affinity and determine whether the lipid disrupts or preserves the HN–BAX interface. The liposome permeabilization assay format used by Guo et al. 2003 and Morris et al. 2019 is the gold-standard functional readout.

- **FEP/MD simulation with explicit lipid bilayer**: Free-energy perturbation or molecular dynamics in an explicit DOPC/CL MOM-mimetic bilayer, with the myristoyl chain pre-inserted, could model BAX groove accessibility and myristoyl anchor geometry in silico — bypassing the AlphaFold blind spot for non-standard chemistry.
- **Cellular BAX translocation assay**: GFP-BAX translocation from cytosol to mitochondria in response to apoptotic stimulus (e.g., staurosporine), with and without myristoyl-HN pre-treatment, would directly test the functional hypothesis in a relevant cellular context.
- **Alternative computational tools**: RosettaLigand, Glide, or AutoDock with explicit myristoyl parameterization could predict the lipid tail placement and BAX groove binding mode — providing the structural data that AlphaFold-based pipelines cannot currently generate for lipidated peptides.

RAW METRICS

Metric	Value
pLDDT	None (no structure generated)
pTM	None
ipTM	None
Binder probability	None
Chai-1 agreement	None (not run)
Heuristic aggregation propensity	0.31 (hotspot residues 7–20)
Heuristic stability score	0.37
Heuristic BBB penetration	0.16
Heuristic half-life estimate	Moderate-to-long (~1–6 hours)

Heuristic metrics are sequence-based estimates of the unmodified Humanin backbone only — not real wet-lab measurements, not reflective of the myristoyl modification, and not predictive of BAX binding.

SEQUENCES

NATIVE

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MAPRGFSCLLLLTSEIDLPVKRRA
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MODIFIED

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Myr-MAPRGFSCLLLLTSEIDLPVKRRA
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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
- FAILED verdict reflects a tool-limit failure (non-standard lipid modification not supported by current AlphaFold-derived pipelines) — not a biological invalidation of the myristoylation hypothesis
- heuristic peptide profile metrics (aggregation, stability, BBB, half-life) are sequence-based estimates of the unmodified Humanin backbone only — they do not reflect the myristoyl modification or BAX engagement
- myristoylation alone may be insufficient for selective mitochondrial outer membrane targeting without a complementary electrostatic second signal — non-specific membrane partitioning is a known risk
- N-terminal myristoylation of Humanin has no experimental precedent in the retrieved literature; all claims about predicted behavior are speculative

CITATIONS

1. **PMID —** (2003) — — Humanin peptide suppresses apoptosis by interfering with Bax activation
2. **PMID —** (2019) — — Humanin induces conformational changes in the apoptosis regulator BAX and sequesters it into fibers, preventing mitochondrial outer-membrane permeabilization
3. **PMID —** (2004) — — Humanin: after the discovery
4. **PMID —** (2005) — — Cytoprotective peptide humanin binds and inhibits proapoptotic Bcl-2/Bax family protein BimEL
5. **PMID —** (2004) — — Unravelling the role of Humanin
6. **PMID —** (2021) — — Humanin: A mitochondrial-derived peptide in the treatment of apoptosis-related diseases
7. **PMID —** (2022) — — Humanin and Alzheimer's disease: The beginning of a new field
8. **PMID —** (2022) — — Cardio-protective role of Humanin in myocardial ischemia-reperfusion

9. **PMID —** (2023) — — Humanin and Its Pathophysiological Roles in Aging: A Systematic Review
10. **PMID —** (2016) — — Humanin: Functional Interfaces with IGF-I
11. **PMID —** (2026) — — Renoprotective Effect of S14G-Humanin on Renal Ischemia/Reperfusion Injury by Activation of STAT3 and ERK 1/2 Signal Transduction Pathways in Rats