DISTILLATION №79 · ALEMBIC LABS

SERMORELIN — MET-27 → L-NORLEUCINE (NLE) SINGLE SUBSTITUTION

generated 2026-05-05T02:59:07.548504+00:00

DISCARDED | PERFORMANCE | MET-27 → L-NORLEUCINE (NLE) SINGLE SUBSTITUTION | GROWTH HORMONE-RELEASING HORMONE RECEPTOR

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
48.9%	0.434 / 0.413	DISCARDED

TARGET	UNIPROT	BINDING PROBABILITY
Growth hormone-releasing hormone receptor	Q02643	—

TLDR

Fold №79 tested whether replacing Met-27 in Sermorelin with L-norleucine (Nle) — a sulfur-free, isosteric hydrophobic residue — could yield an oxidation-resistant scaffold without perturbing GHRHR engagement. The prediction returned pLDDT 0.49 and ipTM 0.41, mirroring the structural tool-limit failures seen across all five prior Sermorelin folds at this lab. This is a DISCARDED fold due to tool-resolution limitations on the GHRHR ECD complex, not a biological invalidation of the Nle substitution concept. The hypothesis remains chemically sound and deserves wet-lab adjudication.

EXECUTIVE SUMMARY

Sermorelin Met-27→Nle: pLDDT 0.49, ipTM 0.41 — tool-limit DISCARD matching all 5 prior Sermorelin folds. The oxidation-resistant scaffold hypothesis is chemically sound; wet-lab binding and oxidative stress assays are the required next step.

DETAILED ANALYSIS

Sermorelin is a 29-residue synthetic GHRH analogue representing the minimal bioactive fragment that retains full agonist activity at the growth hormone-releasing hormone receptor (GHRHR, UniProt Q02643). Its pharmacophore architecture is bipartite: the N-terminal domain (residues 1–10 approximately) drives receptor activation, while the C-terminal segment (roughly LLQDIMSR, positions 22–29) forms an amphipathic α-helix critical for receptor docking and potency. Met-27 sits within this C-terminal pharmacophore and is a well-recognized oxidation liability in GHRH-derived peptides — methionine sulfoxide formation introduces polarity and steric bulk that can disrupt local hydrophobic packing and reduce helical propensity.

The modification hypothesis is among the most conservative possible for this scaffold: a single internal substitution replacing sulfur-containing Met with L-norleucine (Nle), an unbranched C6 aliphatic side chain that is isosteric with Met in linear geometry and hydrophobic volume but lacks the oxidation-prone sulfur atom. Nle has precedent as a Met bioisostere in oxytocin, calcitonin, and parathyroid hormone analogues without loss of receptor affinity. The researcher correctly identified this as the lowest-perturbation modification yet attempted on Sermorelin at this lab — prior folds explored N-terminal Aib substitution (Fold #69), D-Ala at position 2 (Fold #2), lactam stapling (Fold #42), and hexenoyl N-capping (Fold #53), all of which were also DISCARDED with pLDDT clustering between 0.48–0.50.

The structural prediction returned pLDDT 0.49, pTM 0.43, and ipTM 0.41 — values essentially identical to those observed in all previous Sermorelin folds at this lab. This consistency is diagnostically important: it strongly suggests that the tool stack (Boltz-2/Chai-1 on the GHRHR ECD complex) is operating at the resolution floor for this peptide-receptor system, not that the Nle substitution itself is structurally disruptive. A modification as conservative as a single isosteric swap at an internal hydrophobic position should not produce a structurally distinct outcome from the native sequence — and it did not, for better or worse.

The literature context is supportive of the hypothesis in principle. The C-terminal helix of GHRH analogues is a pharmacophore region where hydrophobic packing drives receptor contacts, and Nle preserves the aliphatic character needed for helix stabilization. Studies on Sermorelin sub-fragments (PMID:37688464) confirm backbone stability of the C-terminal octapeptide under enzymatic and serum conditions, and commercial quality audits of gray-market sermorelin products reveal high purity failure rates consistent with chemical degradation — motivating oxidation-resistant scaffold engineering. However, no SAR study has directly addressed Met-27 tolerance in GHRHR, and no co-crystal or cryo-EM structure of sermorelin bound to GHRHR exists to confirm whether the sulfur atom of Met-27 participates in specific receptor contacts that Nle cannot replicate.

Heuristic sequence-based properties of the Nle variant are unremarkable: aggregation propensity 0.119 (low), stability score 0.44 (moderate), half-life

estimate moderate-to-long (1–6 hours). BBB penetration is essentially zero (0.003), consistent with a 29-residue peptide. These heuristics are not derived from the structural prediction and carry their own uncertainty, but they suggest no obvious liabilities introduced by the Nle swap.

The broader pattern across Folds #2, #42, #53, #60, #69, and now #79 is now unambiguous: the current in silico tool stack cannot produce reliable complex-confidence metrics for Sermorelin bound to the GHRHR ECD at the resolution needed to adjudicate single-residue substitutions. The pLDDT ceiling at ~0.49–0.50 across six structurally diverse modifications (D-amino acid, Aib, lactam staple, N-cap, truncation, isosteric swap) indicates a systemic tool-limitation for this peptide-receptor pair, not a series of independently failing modifications. This is a critical lab-level insight.

For the Nle hypothesis specifically, the appropriate path forward is wet-lab validation rather than further in silico iteration at this lab. Competitive binding assays against GHRHR, accelerated oxidation stress studies comparing Met vs. Nle variants, and helical content comparison by CD spectroscopy would directly answer the questions that the current tool stack cannot. The hypothesis is chemically well-founded, literature-supported by analogy, and represents a genuinely useful stability engineering strategy — it simply cannot be adjudicated by the predictors available here.

RESEARCH BRIEF

FOLD №79 — SERMORELIN MET-27 → NORLEUCINE

DISCARDED | TOOL-LIMIT FAILURE

TLDR

Fold №79 is **DISCARDED** due to a tool-limit failure: the Boltz-2/Chai-1 stack returned pLDDT 0.49 and ipTM 0.41 on the Sermorelin[Met27Nle]–GHRHR ECD complex — values that fall below the confidence threshold required to evaluate interface geometry and binding engagement. This is the sixth consecutive Sermorelin fold at this lab to produce pLDDT ≈ 0.48–0.50, establishing a clear systemic tool-resolution ceiling for this peptide–receptor pair rather than a sequence-specific failure of the Nle modification.

WHAT WE TRIED

Met-27 is embedded in Sermorelin's C-terminal pharmacophore segment (LLQDIMSR, residues 22–29), which forms an amphipathic α-helix critical for GHRHR docking. Methionine is highly susceptible to sulfoxide formation under oxidative stress — a known liability in peptide therapeutics that introduces polarity and steric bulk capable of disrupting local hydrophobic packing and reducing helical propensity. Gray-market sermorelin products show purity failure rates up to 71% in commercial audits (DOI:10.20944/preprints202604.1748.v1), a pattern consistent with chemical degradation of the native sequence.

The proposed modification was a single substitution of Met-27 with L-norleucine (Nle): an unbranched C6 aliphatic side chain that is geometrically isosteric with methionine but lacks the sulfur atom entirely. This is the textbook bioisosteric replacement for Met, validated in oxytocin, calcitonin, and parathyroid hormone analogues without receptor affinity loss. The hypothesis was that Nle would preserve the hydrophobic packing of the C-terminal helix while eliminating the oxidation liability — yielding a structurally superimposable complex with comparable or marginally improved interface confidence relative to wild-type Sermorelin. Crucially, this was the most conservative modification yet attempted on Sermorelin at Alembic Labs: a single internal isosteric swap at one hydrophobic position, diverging from the staple (Fold #42), N-cap (Fold #53), Aib substitution (Fold #69), D-amino acid substitution (Fold #2), and truncation (Fold #60) strategies tested previously.

WHY IT WAS DISCARDED

The structural prediction returned:

Metric	Value
pLDDT	0.49
pTM	0.43
ipTM	0.41
Chai-1 agreement	None
Boltz-2 affinity module	No values produced
Binding change prediction	None

These values are below the confidence threshold (pLDDT ≥ 0.70, ipTM ≥ 0.60) required to assess interface geometry, helix integrity, or binding engagement in silico. The Boltz-2 affinity module produced no output, meaning no affinity prediction was generated at all.

Critically, these numbers are essentially identical to those from every prior Sermorelin fold at this lab — Fold #2 (pLDDT 0.49), Fold #42 (pLDDT 0.50), Fold #53 (pLDDT 0.49), Fold #60 (pLDDT 0.48), and Fold #69 (pLDDT 0.48). A modification as conservative as a single isosteric swap at an internal hydrophobic position should not produce a structurally distinct outcome from the native sequence. The fact that it produced identical sub-threshold confidence to all prior structurally diverse modifications confirms this is a **systemic tool-limitation for the Sermorelin–GHRHR ECD complex**, not a sequence-specific failure of the Nle substitution.

WHAT THIS DOESN'T MEAN

DISCARDED does not mean disproved. The current tool stack has demonstrated a consistent inability to produce usable confidence scores for Sermorelin bound to the GHRHR ECD — this is a property of the prediction system, not a verdict on the chemistry. The Met-27 → Nle hypothesis is chemically well-grounded: Nle is an established Met bioisostere with a strong analogy record across multiple peptide therapeutics. The literature supports the biological rationale (oxidation-prone Met in a hydrophobic pharmacophore helix; analogy to calcitonin, oxytocin, and PTH Nle variants), and the heuristic sequence-based stability profile (aggregation propensity 0.119, moderate-to-long half-life estimate) raises no obvious concerns. The question of whether Met-27 → Nle preserves GHRHR binding affinity and improves oxidative stability in sermorelin is **unanswered by this fold**, not answered negatively.

WHAT WOULD ANSWER THE QUESTION

- **Competitive radioligand or fluorescence binding assay (GHRHR-expressing cells):** Direct measurement of Ki or IC50 for Sermorelin[Met27Nle] vs. wild-type Sermorelin against GHRHR. This is the gold-standard SAR assay for the receptor-tolerance question and does not depend on structural prediction confidence.
- **Accelerated oxidative stress study (H_2O_2 or metal-catalyzed oxidation) + LC-MS/MS:** Quantify the rate and extent of Met-27 sulfoxide formation in the native sequence vs. stability confirmation of the Nle variant under physiological oxidative conditions. This directly validates the stability engineering rationale.
- **Circular dichroism (CD) spectroscopy:** Compare helical content of Sermorelin vs. Sermorelin[Met27Nle] in aqueous buffer and in the presence of a membrane-mimetic environment. If the Nle swap preserves helical propensity in the C-terminal pharmacophore, this provides indirect structural support.
- **AlphaFold3 server or Rosetta FoldAndDock with ensemble sampling:** The current tool stack has reached a ceiling for this peptide-receptor pair across six folds. A purpose-built GPCR-peptide docking protocol (e.g., RosettaDock,

HADDOCK with restraints from known GHRH SAR data, or GNIna) using available GHRHR homology models would provide a more informative computational assessment than continuation with Boltz-2/Chai-1 on this target.

RAW METRICS

Parameter	Value
pLDDT	0.489
pTM	0.434
ipTM	0.413
Chai-1 agreement	None
Boltz-2 affinity	Not produced
Predicted binding change	None
Aggregation propensity (heuristic)	0.119 (low)
Stability score (heuristic)	0.44 (moderate)
BBB penetration (heuristic)	0.003 (negligible)
Half-life estimate (heuristic)	Moderate-to-long (~1–6 h)

Heuristic values are sequence-based estimates, not structural or experimental measurements.

Lab pattern note: This is the sixth Sermorelin fold to be DISCARDED with pLDDT 0.48–0.50 (Folds #2, #42, #53, #60, #69, #79). The current tool stack has consistently failed to resolve this peptide–GHRHR complex. Future Sermorelin folds should consider either alternative computational platforms (AlphaFold3, Rosetta, HADDOCK) or direct wet-lab progression rather than further Boltz-2/Chai-1 iteration.

SEQUENCES

NATIVE

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YADAIFTNSYRKVLGQLSARKLLQDIMSR
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MODIFIED

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YADAIFTNSYRKVLGQLSAR KLLQDI(Nle)SR
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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
- pLDDT 0.49 and ipTM 0.41 fall below reliable confidence thresholds — interface geometry and helix integrity cannot be assessed
- no Boltz-2 affinity value was produced; binding engagement is entirely unverified in silico
- six consecutive Sermorelin folds at this lab have returned pLDDT 0.48–0.50, indicating a systemic tool-resolution ceiling for the Sermorelin–GHRHR ECD complex, not fold-specific failures
- heuristic peptide properties (aggregation, stability, half-life) are sequence-based estimates only — not derived from structural prediction or experimental data
- L-norleucine is a non-proteinogenic amino acid; its incorporation requires custom solid-phase synthesis and is not captured by standard sequence-based predictors
- no co-crystal or cryo-EM structure of sermorelin bound to GHRHR exists; structural inference from homology models carries additional uncertainty

CITATIONS

1. **PMID —** (1999) — — Sermorelin: a review of its use in the diagnosis and treatment of children with idiopathic growth hormone deficiency
2. **PMID —** (2023) — — In-house standards derived from doping peptides: Enzymatic and serum stability and degradation profile of GHRP and GHRH-related peptides
3. **PMID —** (2006) — — Sermorelin: a better approach to management of adult-onset growth hormone insufficiency?
4. **PMID —** (2025) — — Growth Hormone-Releasing Hormone Antagonists Increase Radiosensitivity in Non-Small Cell Lung Cancer Cells
5. **PMID —** (2024) — — Antagonist of Growth Hormone-Releasing Hormone Receptor MIA-690 Suppresses the Growth of Androgen-Independent Prostate Cancers
6. **PMID —** (2019) — — Growth Hormone-Releasing Hormone Receptor Antagonist Modulates Lung Inflammation and Fibrosis due to Bleomycin
7. **PMID —** (2026) — — Evaluation of Research Grade Peptides Marketed Directly to Consumers Reveals Extensive Variability in Purity and Measured Abundance
8. **PMID —** (2026) — — Safety and Efficacy of Approved and Unapproved Peptide Therapies for Musculoskeletal Injuries and Athletic Performance

9. **PMID —** (2020) — — Beyond the androgen receptor: the role of growth hormone secretagogues in the modern management of body composition in hypogonadal males

SOLANA SIGNATURE 53whHJMQexCjkT778wawyBDEkbB7R5FLaLRT1Bdmq1kYrVHPhXXT5Euhu1xb3rKvjr1YcTjw1CM9PD3Q2Y43czdZ
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