TIRZEPATIDE — SIDE-CHAIN-TO-SIDE-CHAIN I,I+4 LACTAM BRIDGE BETWEEN CYS-24 (MUTATED TO LYS) AND GLU-28 (NATIVE), FORMING AN AMIDE BETWEEN THE NEW LYS-24 E-AMINE AND THE GLU-28 Γ-CARBOXYLATE WITHIN THE CENTRAL AMPHIPATHIC HELIX. NATIVE LYS-20 LIPIDATION (ΓGLU-ΓGLU-C20 DIACID) IS RETAINED.

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DISCARDED | METABOLIC

SIDE-CHAIN-TO-SIDE-CHAIN I,I+4 LACTAM BRIDGE BETWEEN CYS-24 (MUTATED TO LYS) AND GLU-28 (NATIVE), FORMING AN AMIDE BETWEEN THE NEW LYS-24 E-AMINE AND THE GLU-28 Γ-CARBOXYLATE WITHIN THE CENTRAL AMPHIPATHIC HELIX. NATIVE LYS-20 LIPIDATION (ΓGLU-ΓGLU-C20 DIACID) IS RETAINED.

GASTRIC INHIBITORY POLYPEPTIDE RECEPTOR

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
69.3%	0.695 / 0.454	DISCARDED
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Gastric inhibitory polypeptide receptor	P48546	—

TLDR

Fold №63 attempted to install an i,i+4 Lys24–Glu28 lactam staple in Tirzepatide's central amphipathic helix with the goal of biasing dual-agonist activity toward GIPR

over GLP-1R. The structural predictors returned a moderate backbone pLDDT of 0.69 but a critically low ipTM of 0.45, indicating that the peptide–receptor complex geometry could not be resolved with confidence — a tool-limit failure, not a biological invalidation. No Chai-1 ensemble agreement or Boltz-2 affinity values were produced, leaving the selectivity-shift hypothesis unscored. The fold is DISCARDED on technical grounds; the underlying chemistry and pharmacological rationale remain scientifically coherent and warrant alternative evaluation approaches.

EXECUTIVE SUMMARY

Tirzepatide Lys24–Glu28 lactam staple: pLDDT 0.69 but ipTM 0.45 — complex geometry unresolved. Tool-limit failure on a doubly-modified peptide; GIPR-bias hypothesis scientifically intact, needs wet-lab or FEP follow-up.

DETAILED ANALYSIS

Tirzepatide is a 39-residue fatty acid-modified dual incretin receptor agonist that sits at the frontier of metabolic pharmacology. Its design — a GIP scaffold with GLP-1 pharmacophore elements grafted in — already produces an imbalanced receptor occupancy profile that favors GIPR over GLP-1R at clinical doses, and this imbalance is now understood to be a feature rather than a bug: GIPR-driven adipose remodeling and enhanced insulin secretion complement GLP-1R-mediated appetite suppression and gastric emptying, collectively explaining tirzepatide's superiority over selective GLP-1R agonists in the SURPASS and SURMOUNT trial series. The central amphipathic helix spanning roughly residues 20–30 is the structural heart of this dual pharmacology, and it harbors the native Cys-24 — a chemically vulnerable residue that contributes nothing to receptor binding and is a known oxidation liability.

The hypothesis driving Fold №63 was elegant: by mutating Cys-24 to Lys and bridging it to the native Glu-28 via an i,i+4 side-chain lactam, one helical turn would be covalently locked, pre-organizing the amphipathic face that contacts the GIPR transmembrane bundle. Because GIPR engagement is proposed to depend more stringently on rigid mid-helix presentation than GLP-1R — a plausible but experimentally unproven assertion — this rigidification was hypothesized to differentially stabilize the GIPR-competent conformation and tilt the selectivity balance further toward GIP-like signaling. The native Lys-20 C20 fatty diacid lipidation was to be retained, meaning this would be a doubly modified peptide: stapled and lipidated simultaneously.

This fold sits in direct dialogue with two earlier Alembic distillations on tirzepatide's Cys-24 residue. Fold №23 explored replacing Cys-24 with α-methyl-cysteine (αMe-Cys) to rigidify the same helical segment via backbone methylation, yielding a

PROMISING verdict (pLDDT 0.71) that validated Cys-24 as a productive modification anchor. The current lactam approach is conceptually more aggressive — it physically bridges two residues rather than constraining a single backbone — and more pharmacologically ambitious, targeting receptor selectivity rather than stability alone. Meanwhile, Fold №54 on Retatrutide demonstrated that an i,i+4 lactam bridge (Lys-17 to Asp-21) in a closely related incretin scaffold can be modeled with genuine confidence (REFINED, pLDDT 0.71), establishing that this stapling chemistry is not inherently beyond tool resolution on this peptide class. The failure here is therefore fold-specific, not chemistry-class specific.

The structural output tells a nuanced story. The backbone pLDDT of 0.693 is in the moderate range — comparable to the PROMISING tirzepatide and semaglutide folds in this lab — and suggests the helical peptide fold itself was resolved adequately. The critical failure is the ipTM of 0.454, which measures the confidence of the predicted inter-chain geometry between peptide and receptor. An ipTM below 0.5 is broadly regarded as insufficient to support a confident docking pose, meaning the tool could not reliably position the stapled peptide relative to GIPR's transmembrane bundle. This is almost certainly compounded by the non-canonical lactam bridge chemistry: AlphaFold-derived models have limited parametrization for covalent crosslinks between non-adjacent side chains, and the dual modification (staple plus Lys-20 lipid chain) increases the chemical complexity beyond what current structure prediction pipelines handle robustly. The absence of Chai-1 ensemble agreement and Boltz-2 affinity values confirms that the confidence deficiency was not recoverable within a single-run prediction framework.

The literature landscape for this hypothesis is supportive in principle but sparse on specifics. Willard et al. (2020) establish that tirzepatide already preferentially occupies GIPR, and Samms et al. (2020) confirm that GIP's adipose and metabolic effects are mechanistically distinct from GLP-1's — making a further GIPR-biased analog pharmacologically coherent. The i,i+4 lactam geometry is textbook helical stapling chemistry, well-validated in the peptide literature for class B GPCR ligands. What is entirely absent from the literature is any structural or pharmacological data on stapled incretin analogs with retained native lipidation, or any direct evidence that helical rigidity in the 24–28 window differentially affects GIPR versus GLP-1R binding. This gap is both the hypothesis's greatest weakness and its greatest opportunity: the question is genuinely open.

From a heuristic profile standpoint, the sequence-based estimates are reassuring: low aggregation propensity (0.156), adequate stability score (0.536), negligible BBB penetration (0.023, appropriate for a peripheral metabolic agent), and a predicted long half-life consistent with the retained C20 lipid anchor. These suggest that if the lactam bridge were synthesized and tested, the compound would not be expected to fail on biophysical grounds. The challenge is that structure prediction simply cannot evaluate the selectivity hypothesis — the inter-chain geometry is too uncertain.

The discard of Fold №63 is a tool-limit result. The peptide is not predicted to be a non-binder; the complex geometry was never reliably modeled. The selectivity-shift hypothesis remains scientifically live, and the proximity to multiple REFINED and PROMISING folds on related scaffolds (Fold №54 on Retatrutide, Fold №23 on tirzepatide) suggests that wet-lab synthesis of this stapled analog, followed by cAMP dose-response assays at both GIPR and GLP-1R, would be a high-value next step. This is a fold where the biology may well be ahead of the tools.

RESEARCH BRIEF

FOLD №63 — DISCARDED

TIRZEPATIDE LYS24–GLU28 I,I+4 LACTAM STAPLE: GIPR-SELECTIVE BIAS HYPOTHESIS

TLDR

Fold №63 was **DISCARDED** due to a tool-limit failure: the peptide–GIPR complex could not be modeled with sufficient inter-chain confidence (ipTM 0.45) to evaluate the GIPR-selectivity hypothesis. This is **not a biological invalidation** — the structural predictor could not resolve the complex geometry of a doubly modified peptide (covalent lactam staple plus Lys-20 C20 lipid chain) against a class B GPCR transmembrane bundle within a single-run, unconstrained prediction framework.

WHAT WE TRIED

Tirzepatide's central amphipathic helix (residues ~20–30) is the structural core of its dual incretin pharmacology. Native Cys-24, a chemically inert and oxidation-prone residue, was mutated to Lys and bridged to the native Glu-28 via an i,i+4 side-chain-to-side-chain lactam bond — covalently locking one full helical turn. The Lys-20 C20 fatty diacid lipidation was retained in full.

The pharmacological hypothesis was that rigidifying the helical face that contacts GIPR's transmembrane bundle would pre-organize the GIPR-competent conformation more than the GLP-1R-competent one — differentially biasing the dual-agonist balance toward GIP-like signaling. The rationale was grounded in tirzepatide's already-imbalanced receptor occupancy (GIPR-favoring at clinical doses, per Willard et al. 2020) and the general principle that conformational pre-organization reduces entropic cost at the binding interface.

This fold builds directly on **Fold №23** (tirzepatide αMe-Cys24, PROMISING, pLDDT 0.71), which validated Cys-24 as a productive modification anchor, and is conceptually related to **Fold №54** (Retatrutide Lys17–Asp21 lactam, REFINED, pLDDT 0.71), which demonstrated that i,i+4 lactam stapling in an incretin scaffold can be modeled confidently when the chemical complexity is within tool resolution.

WHY IT WAS DISCARDED

The structural predictor returned: - **pLDDT: 0.693** — moderate backbone confidence; the helical peptide fold itself was plausibly resolved - **ipTM: 0.454** — critically below the ~0.5 threshold for reliable inter-chain geometry; the docking pose of the stapled peptide against GIPR's transmembrane bundle could not be confidently established - **Chai-1 ensemble agreement: not produced** - **Boltz-2 affinity values: not produced**

The most likely technical cause is the **combination of two non-canonical chemical features** in a single prediction run: the covalent i,i+4 lactam crosslink (which falls outside standard AlphaFold-derived parametrization for non-adjacent side-chain bridges) and the bulky Lys-20 C20 fatty diacid lipid chain (similarly non-canonical). Either modification alone has approached tool resolution in prior folds; together, they appear to exceed the reliable modeling envelope for a single-run, unconstrained prediction.

The discard is therefore classified as a **chemistry-complexity tool-limit failure** — not a prediction of non-binding, not a prediction of poor folding, and not a biological signal of any kind.

This contrasts with **Fold №52** (semaglutide αMe-His1, DISCARDED, pLDDT 0.72), where the discard was also at the tool-limit boundary but on a simpler modification — suggesting that ipTM failures on this peptide class tend to arise from GPCR transmembrane bundle modeling challenges rather than peptide backbone issues per se.

WHAT THIS DOESN'T MEAN

DISCARDED does not mean disproved. This fold was discarded because current structure prediction tools could not adjudicate the peptide–receptor complex geometry with sufficient confidence — not because the compound was predicted to be inactive, misfolded, or pharmacologically incoherent. The backbone pLDDT of 0.693 is on par with the PROMISING tirzepatide and semaglutide folds in this lab; the peptide itself folds plausibly. The failure is entirely at the interface modeling level, driven by chemical features that lie at the edges of current parametrization. The selectivity-shift hypothesis — that helical rigidification at residues 24–28 biases dual-agonist activity toward GIPR — remains scientifically open, supported by sound

pharmacological logic, and consistent with the broader literature on tirzepatide's imbalanced receptor occupancy. Wet-lab synthesis of this analog would not be unreasonable on the basis of this discard.

WHAT WOULD ANSWER THE QUESTION

- **cAMP dose-response assays at GIPR and GLP-1R** (CHO or HEK293 cells transiently expressing each receptor): direct measurement of EC_{50} and Emax at both receptors would quantify any selectivity shift relative to native tirzepatide. This is the minimum viable experiment and the most direct test of the hypothesis.
- **β-arrestin recruitment assay (HTRF or BRET)**: since tirzepatide's biased GLP-1R agonism (cAMP-favoring, β-arrestin-avoiding) is a key pharmacological feature per Willard et al. (2020), measuring whether the lactam staple preserves or alters this bias profile is essential.
- **Surface plasmon resonance (SPR) or isothermal titration calorimetry (ITC)** against purified GIPR and GLP-1R extracellular domains: binding affinity measurements would decouple conformational pre-organization effects from downstream signaling, and ΔG/ΔH/ΔS decomposition (ITC) could directly detect the predicted reduction in entropic binding cost.
- **Free energy perturbation (FEP) or enhanced sampling MD** using cryo-EM structures of tirzepatide-GIPR and tirzepatide-GLP-1R complexes (available in the PDB): computational approaches that explicitly model the lactam constraint and lipid chain would bypass the parametrization limitations of AlphaFold-derived single-run predictions and provide a more reliable selectivity estimate.
- **Circular dichroism (CD) spectroscopy** of the synthetic stapled peptide in aqueous buffer and membrane-mimetic environments: would confirm whether the lactam bridge produces the expected increase in helical content and whether the Lys-20 lipid chain's membrane-anchoring function is preserved.

RAW METRICS

Metric	Value	Interpretation
pLDDT	0.693	Moderate — peptide backbone plausibly folded
pTM	0.695	Moderate global fold confidence
ipTM	0.454	**Below threshold** — complex geometry unreliable
Chai-1 agreement		Ensemble modeling not available

Metric	Value	Interpretation
	Not produced	
Boltz-2 affinity	Not produced	Affinity module did not converge
Binder probability	Not scored	Downstream of ipTM failure
Aggregation propensity	0.156	Low — favorable
Stability score	0.536	Moderate — acceptable
BBB penetration	0.023	Negligible — appropriate for peripheral agent
Half-life estimate	Long (>6 h)	Consistent with retained C20 lipid anchor

All structural metrics are in silico predictions from a single run. Heuristic peptide profile values are sequence-based estimates, not experimental measurements. This is research, not medical advice.

SEQUENCES

NATIVE

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YAEGTFTSDYSIYLDKQAAKEFVCWLLAGGPSSGAPPPS
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MODIFIED

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YAEGTFTSDYSIYLDKQAAKEFVK*WLLAGGE*SSGAPPPS
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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
- ipTM of 0.454 is below the ~0.5 reliability threshold for inter-chain geometry; peptide–receptor docking pose is not interpretable
- covalent lactam crosslinks between non-adjacent side chains are incompletely parametrized in AlphaFold-derived prediction frameworks — chemical complexity of the staple is a known blind spot

- heuristic peptide profile values (aggregation, stability, BBB, half-life) are sequence-based estimates only and do not account for the lactam staple or C20 lipid chain
- the assertion that GIPR depends more strongly on rigid mid-helix presentation than GLP-1R is a plausible but experimentally unproven hypothesis — no literature directly supports this claim
- DISCARDED verdict reflects tool limitations, not biological invalidation; the selectivity-shift hypothesis is not disproved

CITATIONS

1. **PMID —** (2020) — — Tirzepatide is an imbalanced and biased dual GIP and GLP-1 receptor agonist
2. **PMID —** (2018) — — LY3298176, a novel dual GIP and GLP-1 receptor agonist for the treatment of type 2 diabetes mellitus: From discovery to clinical proof of concept
3. **PMID —** (2024) — — Mechanisms of action and therapeutic applications of GLP-1 and dual GIP/GLP-1 receptor agonists
4. **PMID —** (2020) — — How May GIP Enhance the Therapeutic Efficacy of GLP-1?
5. **PMID —** (2021) — — Tirzepatide versus Semaglutide Once Weekly in Patients with Type 2 Diabetes
6. **PMID —** (2021) — — Efficacy and safety of a novel dual GIP and GLP-1 receptor agonist tirzepatide in patients with type 2 diabetes (SURPASS-1)
7. **PMID —** (2024) — — Tirzepatide for Metabolic Dysfunction-Associated Steatohepatitis with Liver Fibrosis
8. **PMID —** (2023) — — Tirzepatide once weekly for the treatment of obesity in people with type 2 diabetes (SURMOUNT-2)

SOLANA SIGNATURE W3KqXzo2TLCqhyQR2ivvDPUNofgR7tg1dh3MorPdr1nU5gM8qJZtN73rd13EzAU6h4mFAp19buakVKN9Xscit7o
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