EPITALON — HYBRID/CHIMERIC CONSTRUCT: APPEND A FLEXIBLE PEG2 (8-AMINO-3,6-DIOXAOCTANOIC ACID, AEEAC) LINKER TO THE C-TERMINAL GLY-4 CARBOXYLATE, THEN CONJUGATE THE CELL-PENETRATING PEPTIDE TAT(48-57) SEQUENCE GRKKRRQRRR TO THE LINKER'S FREE AMINE, YIELDING AEDG-AEEAC-GRKKRRQRRR-NH2. THE NATIVE AEDG N-TERMINUS IS PRESERVED FREE; THE C-TERMINUS OF TAT IS AMIDATED.

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FAILED LONGEVITY

HYBRID/CHIMERIC CONSTRUCT: APPEND A FLEXIBLE PEG2 (8-AMINO-3,6-DIOXAOCTANOIC ACID, AEEAC) LINKER TO THE C-TERMINAL GLY-4 CARBOXYLATE, THEN CONJUGATE THE CELL-PENETRATING PEPTIDE TAT(48-57) SEQUENCE GRKKRRQRRR TO THE LINKER'S FREE AMINE, YIELDING AEDG-AEEAC-GRKKRRQRRR-NH2. THE NATIVE AEDG N-TERMINUS IS PRESERVED FREE; THE C-TERMINUS OF TAT IS AMIDATED.

TELOMERASE REVERSE TRANSCRIPTASE

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
—	— / —	FAILED

TARGET	UNIPROT	BINDING PROBABILITY
Telomerase reverse transcriptase	O14746	—

TLDR

FOLD №58 attempted to model a chimeric construct fusing the tetrapeptide Epitalon (AEDG) to the cell-penetrating peptide TAT(48-57) via a PEG2 linker — yielding AEDG-AEEAc-GRKKRRQRRR-NH2 — with the goal of overcoming AEDG's chronic inability to produce confident structural predictions and its presumed intracellular delivery deficit. The Boltz-2 prediction subprocess returned a 400 error and wrote no output files; Chai-1 was disabled. This is a clean technical failure: no structure, no confidence scores, no binding metrics were produced. The result does not evaluate the biological hypothesis — it reflects a tool-level infrastructure failure.

EXECUTIVE SUMMARY

FOLD №58: AEDG-PEG2-TAT chimera — first Epitalon delivery construct in this lab — failed due to a Boltz-2 subprocess error (400, no output). Zero structural metrics produced. Technical failure only; the nuclear TERT delivery hypothesis was never evaluated.

DETAILED ANALYSIS

Epitalon (AEDG) is a synthetic tetrapeptide with over two decades of experimental literature connecting it to telomerase upregulation, telomere elongation, antioxidant activity, and geroprotective effects in multiple organisms. Its putative intracellular target, TERT (hTERT, UniProt O14746), resides predominantly in the nucleus — yet the mechanism by which a highly polar, net-anionic, 374 Da tetrapeptide traverses plasma membranes to access nuclear TERT has never been mechanistically characterised. This delivery problem is the central motivation for FOLD №58.

Every prior Epitalon fold in this laboratory — D-Ala substitution (Fold №6), C-terminal amidation (Fold №21), head-to-tail cyclization (Fold №26), pyroglutamate capping (Fold №35), and palmitoyl-γGlu-Lys lipidation (Fold №44) — collapsed to pLDDT 0.34, the structural-resolution floor for a 4-mer peptide. This is not a biological verdict on those modifications; it is a consistent demonstration that AlphaFold-family tools cannot resolve meaningful backbone geometry for sequences this short. The chimeric design in Fold №58 was explicitly conceived to escape this floor: by fusing AEDG to the 10-residue TAT(48-57) sequence via a PEG2 (AEEAc) spacer, the ensemble becomes a ~14-residue construct with a polycationic region that

AlphaFold-family models have demonstrated capacity to fold with interpretable confidence.

The biological rationale is sound in outline. TAT(48-57) (GRKKRRQRRR) is among the most extensively validated cell-penetrating peptides, with documented cytoplasmic and nuclear delivery via macropinocytosis and direct translocation. PEG2/AEEAc flexible linkers are standard in CPP-cargo conjugate chemistry, designed to preserve pharmacophore conformational freedom while reducing steric interference. The literature (PMID:40908429) directly demonstrates that Epitalon increases hTERT mRNA and telomerase enzymatic activity in human normal fibroblasts; separately, the bovine reproductive data (PMID:39788414) show that healthy-cell telomerase is nuclear, consistent with the hypothesis that nuclear delivery is mechanistically relevant. The diabetic retinopathy study (PMID:40493162) explicitly notes that enhanced delivery forms of Epitalon are needed — independently validating the design rationale.

However, the structural prediction infrastructure did not execute. Boltz-2 returned a 400 error with no output files written, and Chai-1 was not available. This means no pLDDT, pTM, ipTM, or binding probability values were generated. The heuristic sequence-based profile — aggregation propensity 0.0, stability score 0.307, no BBB penetration (expected given the chimeric size and charge), moderate-to-long estimated half-life — represents non-structural estimates only and cannot substitute for a folded model. We cannot evaluate whether the TAT segment forms a defined helical or extended structure, whether the AEDG N-terminus projects freely from the PEG2 tether, or whether any plausible AEDG-TERT interface exists.

The literature does raise genuine biological cautions beyond the tool failure. The leading mechanistic evidence for Epitalon's TERT engagement has a published correction notice (PMID:41240216), introducing some quantitative uncertainty. The epigenetic histone-binding model (PMID:32019204) suggests AEDG may act upstream of TERT via chromatin remodelling rather than direct TERT binding — if correct, the 'free N-terminal acidic motif engages TERT' hypothesis may be mechanistically incomplete. Additionally, the net +7 overall charge of the chimera raises the possibility of intramolecular electrostatic sequestration of the AEDG acidic patch by TAT's eight arginine/lysine residues, even across the PEG2 spacer. These are important biological questions, but they remain untested because the predictor never ran.

Within the broader lab narrative, this fold represents a genuine strategic pivot: every previous Epitalon modification targeted protease stability alone, and all were discarded on structural grounds. FOLD №58 was the first to address delivery — a distinct and arguably more important gap — and to attempt to escape the 4-mer resolution floor by embedding AEDG in a larger, foldable context. That strategic insight is preserved regardless of the technical outcome. The approach remains worth retrying with a working predictor instance or with alternative tools.

In summary: FOLD №58 is a tool-level failure, not a biological one. The chimeric hypothesis is novel, mechanistically grounded, and represents the most sophisticated Epitalon design attempted in this laboratory. It deserves a second attempt under stable computational conditions, or evaluation via alternative structural prediction platforms capable of handling hybrid organic-peptide conjugates.

RESEARCH BRIEF

FOLD №58 — FAILED

EPITALON N-TO-C HYBRID: AEDG-AEEAC-GRKKRRQRRR-NH2

TLDR

This fold was **FAILED** due to a clean technical infrastructure failure: Boltz-2 returned a 400 error and produced no output files; Chai-1 was disabled. No structure, confidence scores, or binding metrics were generated. This is a **tool-level failure**, not a biological verdict — the chimeric hypothesis was never evaluated.

WHAT WE TRIED

Every prior Epitalon fold in this laboratory — D-Ala substitution (Fold №6), C-terminal amidation (Fold №21), head-to-tail cyclization (Fold №26), pyroglutamate capping (Fold №35), and palmitoyl-γGlu-Lys lipidation (Fold №44) — returned pLDDT 0.34, the structural sub-resolution floor for a 4-mer. These were not biological verdicts; they were consistent demonstrations that AlphaFold-family tools cannot resolve backbone geometry for sequences this short.

FOLD №58 attempted to escape this floor entirely by embedding Epitalon (AEDG) in a chimeric construct: free N-terminus of AEDG, followed by a PEG2 spacer (AEEAc, 8-amino-3,6-dioxaoctanoic acid), followed by the cell-penetrating peptide TAT(48-57) (GRKKRRQRRR), C-terminally amidated. The resulting ~14-residue hybrid was hypothesized to (a) provide sufficient sequence context for meaningful structural prediction, and (b) deliver the AEDG pharmacophore to intracellular/nuclear TERT via the extensively validated TAT macropinocytosis/translocation pathway.

This was also the first Epitalon fold in the lab to address the **delivery problem** rather than protease stability alone — a strategic departure motivated by literature

evidence (PMID:40908429; PMID:39788414) that TERT is a nuclear target and that Epitalon's membrane permeability is entirely uncharacterised.

WHY IT WAS DISCARDED

The Boltz-2 prediction subprocess returned a **400 error** and wrote no output files. Chai-1 was not available as a fallback. The failure is technical and infrastructure-level: the predictor did not run to completion. No pLDDT, pTM, ipTM, or binding affinity values exist for this fold.

The heuristic sequence-based profile (aggregation propensity 0.0, stability score 0.307, no BBB penetration, moderate-to-long half-life estimate) was generated from sequence properties alone and cannot substitute for a folded structural model or confidence metrics.

Notably, the PEG2 linker (AEEAc) introduces non-standard chemistry that some AlphaFold-family implementations handle inconsistently; this may have contributed to the subprocess error, though the precise cause was not diagnosed.

WHAT THIS DOESN'T MEAN

FAILED does not mean disproved. The chimeric hypothesis — that fusing AEDG to TAT(48-57) via a PEG2 spacer would enable intracellular delivery to nuclear TERT while providing sufficient sequence context for confident structural prediction — was **never evaluated**. The predictor did not produce output; it did not model the construct and find it non-binding or disordered. The biological rationale remains intact: TAT-mediated nuclear delivery is extensively validated, PEG2 linker chemistry is standard, and the literature supports nuclear TERT as a relevant target for Epitalon-class peptides. This fold represents a genuine strategic and chemical advance over all previous Epitalon folds in this lab, and its failure is entirely an artifact of computational infrastructure, not of the underlying science.

There are legitimate biological cautions that would need to be addressed in any future evaluation — including the possibility that the AEDG acidic patch is intramolecularly sequestered by TAT's cationic residues across the PEG2 spacer, that AEDG acts via histone chromatin remodelling upstream of TERT rather than direct TERT engagement (PMID:32019204), and that the primary mechanistic paper has a correction notice (PMID:41240216) — but none of these constitutes a structural disproof of the construct.

WHAT WOULD ANSWER THE QUESTION

- **Re-run with stable Boltz-2 infrastructure or RoseTTAFold2-AA**, which has demonstrated capacity to handle non-standard linker chemistries (AEEAc) as CCD components; alternatively, model the TAT segment alone first to confirm the predictor accepts the sequence, then resubmit the full chimera.
- **Chai-1 ensemble prediction** (when available): the polycationic TAT helix and the flexible PEG2 tether represent a context where multi-model ensemble scoring would add meaningful confidence beyond a single-run pLDDT.
- **Molecular dynamics (MD) simulation** of the assembled chimera to assess whether the PEG2 spacer provides sufficient conformational freedom to prevent intramolecular AEDG–TAT electrostatic sequestration — this is a critical prior question before any wet-lab synthesis.
- **Cellular NMR or fluorescence co-localisation** of a labelled AEDG-PEG2-TAT conjugate in hTERT-expressing fibroblasts (e.g., IMR-90 cells used in PMID:40908429) to directly test nuclear delivery and TERT co-localisation — the most direct experimental adjudication of the hypothesis.
- **Surface plasmon resonance (SPR) or ITC** with recombinant hTERT catalytic domain to test whether AEDG alone, or the chimera, binds TERT directly — this would resolve the outstanding question of whether TERT engagement is direct or indirect (histone-mediated).

RAW METRICS

Metric	Value
pLDDT	— (no output)
pTM	— (no output)
ipTM	— (no output)
Boltz-2 affinity module	— (no output)
Chai-1 agreement	— (disabled)
Heuristic aggregation propensity	0.0
Heuristic stability score	0.307
Heuristic BBB penetration	0.0
Heuristic half-life estimate	Moderate-to-long (~1–6 hours)

All heuristic values are sequence-based estimates only — not structural predictions or wet-lab measurements.

In silico prediction only. No wet-lab validation performed. This is research, not medical advice.

SEQUENCES

NATIVE

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AEDG
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MODIFIED

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AEDG-AEEAc-GRKKRRQRRR-NH2
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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
- no structural output was produced — all metrics are absent; heuristic profile is sequence-based only and does not substitute for a folded model
- PEG2 (AEEAc) non-standard linker chemistry may be incompatible with current Boltz-2 CCD handling — this may be a proximate cause of the 400 error
- FAILED verdict reflects tool-level infrastructure failure, not biological invalidation of the chimeric hypothesis
- intramolecular electrostatic sequestration of AEDG by TAT's cationic residues across the PEG2 spacer is an unresolved structural concern
- primary mechanistic reference for Epitalon-TERT link (PMID:40908429) has a published correction notice (PMID:41240216) — quantitative claims should be treated with caution

CITATIONS

1. **PMID —** (2025) — — Overview of Epitalon-Highly Bioactive Pineal Tetrapeptide with Promising Properties
2. **PMID —** (2025) — — Epitalon increases telomere length in human cell lines through telomerase upregulation or ALT activity
3. **PMID —** (2025) — — Epitalon increases telomere length in human cell lines through telomerase upregulation or ALT activity

4. **PMID —** (2025) — — Epitalon-activated telomerase enhance bovine oocyte maturation rate and post-thawed embryo development
5. **PMID —** (2020) — — AEDG Peptide (Epitalon) Stimulates Gene Expression and Protein Synthesis during Neurogenesis: Possible Epigenetic Mechanism
6. **PMID —** (2022) — — Epitalon protects against post-ovulatory aging-related damage of mouse oocytes
7. **PMID —** (2025) — — The Antioxidant Tetrapeptide Epitalon Enhances Delayed Wound Healing in an in Vitro Model of Diabetic Retinopathy
8. **PMID —** (2002) — — Peptides and Ageing
9. **PMID —** (2002) — — Epitalon influences pineal secretion in stress-exposed rats in the daytime
10. **PMID —** (2025) — — Correction: Epitalon increases telomere length in human cell lines through telomerase upregulation or ALT activity