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In Silico Design of a Multimodal Trimeric Peptide Candidate Predicted to Simultaneously Target Glycemic Control, Autoimmunity, and Pancreatic β-Cell Regeneration in Type 1 Diabetes

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Short Report

Keywords: Type 1 diabetes mellitus, In silico peptide design, Trimeric biopharmaceutical, Multimodal immunotherapy

Posted Date: February 25th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8951973/v1

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Additional Declarations: The authors declare no competing interests.

Abstract

Introduction: Type 1 diabetes mellitus (T1DM) currently affects 9.5 million individuals worldwide, with incidence rising through 2040. Clinical management remains anchored to exogenous insulin replacement, which neither arrests autoreactive $CD8^+$ T-cell–mediated insulitis, nor blocks IL-1β–driven β-cell apoptosis, nor restores PDX-1/NGN3-dependent endocrine identity. No approved agent addresses these three pathological axes concurrently.

Objective: To computationally design and validate TrimInsulinX-3 (TIX-3), a trimeric peptide candidate engineered to simultaneously engage the insulin receptor (InsR), the IL-1R1/IL-1βcomplex, and the GLP-1 receptor/PDX-1 axis within a single molecular scaffold.

Methods: Three functionally orthogonal monomers M1-InsA, M2-ImmunoQ, and M3-BetaR, were de novo designed via RFdiffusion backbone generation, ProteinMPNN sequence optimization, and AlphaFold2 multimer validation, using exclusively open-source platforms. The trimeric construct was assembled with disulfide-stabilized PEG_4 linkers and subjected to 200 ns molecular dynamics (GROMACS 2023.3/AMBER ff19SB). Docking was performed with AutoDock Vina 1.2.7 and cross-validated with HADDOCK 2.4; binding free energies were computed via gmx_MMPBSA. ADMET properties were predicted using pkCSM and SwissADME; immunogenicity was assessed with NetMHCpan-4.1/NetMHCIIpan-4.3.

Results: All monomers adopted stable helix–loop–helix folds (pLDDT > 85; iBSA > 850 $Å^2$). Backbone RMSD stabilized below 2.3 Å over 200 ns simulations. Docking convergence was high across platforms (Cα RMSD < 1.6 Å), with predicted ΔGbind of −11.2, −10.6, and −11.9 $kcal \cdot mol^{-1}$ for InsR, IL-1R1, and GLP-1R, respectively. The predicted ADMET profile, t½ ~18 h, subcutaneous bioavailability ~78%, LogP −1.4, negative AMES mutagenicity, is consistent with injectable peptide therapeutics. After iterative deimmunization, no strong MHC binders were identified across 166 alleles.

Conclusion:

TIX-3 is the first *in silico*–conceived trimeric peptide designed to address the metabolic, immunological, and regenerative axes of T1DM within a unified molecular architecture, and its predicted pharmacological profile supports advancement to experimental validation.

INTRODUCTION

Classified as a chronic autoimmune disorder, type 1 diabetes mellitus (T1DM) currently affects approximately 9.5 million individuals worldwide. The epidemiological landscape demonstrates a substantial rise in disease incidence across all age demographics, with current projections indicating that this upward trajectory is anticipated to accelerate through 2040.[1] Despite more than a century of therapeutic advancement, clinical management remains fundamentally contingent upon exogenous insulin replacement therapy. This intervention addresses the metabolic sequelae of β-cell failure without,

however, modifying the underlying etiopathogenesis, arresting the progression of the autoimmune assault, or regenerating the lost endocrine parenchyma.[2]

The pathology of T1DM exhibits a tripartite framework. Initially, a dysfunction in central and peripheral immune tolerance enables islet-reactive $CD8^+$ T lymphocytes to evade thymic selection and infiltrate the pancreatic islets; a recently identified subpopulation of stem cell-like progenitor cells within this autoreactive compartment continuously replenishes short-lived cytotoxic effectors through a perforin/granzyme B-dependent mechanism, thereby sustaining β-cell destruction well beyond the onset of clinical symptoms.[3] Subsequently, M1-polarized macrophages exacerbate insulitis by secreting IL-1β, which directly promotes β-cell apoptosis via NF-κB-dependent nitric oxide production.[4] Finally, once β-cell mass declines below the threshold requisite for glycemic homeostasis, no currently approved therapeutic intervention can restore endogenous insulin secretion, as the transcriptional machinery governing β-cell identity, centered on the master regulators PDX-1 and NGN3, remains epigenetically silenced in the absence of targeted pharmacological modulation.[5]

Each of these three pathological pillars has independently attracted candidate disease-modifying interventions. The approval of teplizumab, a humanized anti-CD3 monoclonal antibody, by the U.S. Food and Drug Administration (FDA) in 2022 demonstrated that clinical attenuation of the autoimmune process is achievable, delaying progression to stage 3 T1DM by a median of 32.5 months in high-risk individuals through preservation of residual C-peptide secretion.[6] Within the regenerative axis, GLP-1 receptor agonists have demonstrated the capacity to upregulate PDX-1 and NGN3 expression in pancreatic progenitors, combining partial agonism with induction of neogenesis in murine models.[7] In the domain of insulin signaling, computationally optimized peptide agonists have recently been shown to activate the insulin receptor via an extracellular binding conformation distinct from that of native insulin, yielding prolonged glycemic control while preserving metabolic selectivity.[8] Nevertheless, these advances remain confined to discrete molecular scaffolds, and their sequential or combinatorial application introduces cumulative pharmacokinetic complexity, heightened risk of drug-drug interactions, and excessive polypharmacy, factors that collectively impede clinical translation.[9]

The rational design of multifunctional peptides capable of engaging independent receptor systems through structurally autonomous modules, an approach already validated in oncology via bispecific and trispecific antibody platforms, has not yet been systematically explored in the convergent pathogenesis of T1DM.[10] In this study, we describe the *in silico* development of TrimInsulinX-3 (TIX-3), a trimeric peptide candidate engineered from three functionally orthogonal monomeric modules, linked via disulfide-stabilized PEG_4 spacers: M1-InsA, a computationally optimized partial agonist of the insulin receptor; M2-ImmunoQ, a rationally designed inhibitor intended to disrupt the maintenance of autoreactive $CD8^+$ T-cell progenitors through targeted interruption of the IL-1β/IL-17 signaling axis; and M3-BetaR, a bifunctional module engineered to upregulate PDX-1/NGN3 expression while concurrently acting as a partial agonist of the GLP-1 receptor.

METHODS

Target selection and receptor preparation

Three pharmacological targets central to the tripartite pathology of T1DM were selected: the insulin receptor ectodomain (InsR; UniProt P06213; PDB: 6PXV, 2.9 Å), the IL-1 receptor type I/IL-1β complex (IL-1R1; UniProt P14778; PDB: 4DEP, 2.7 Å), and the GLP-1 receptor transmembrane domain (GLP-1R; UniProt P43220; PDB: 6X1A, 3.0 Å). The PDX-1 DNA-binding domain (UniProt P52945; PDB: 2H1K, 2.1 Å) was incorporated as a secondary target for the M3-BetaR module. All structures were retrieved from the RCSB Protein Data Bank (rcsb.org; accessed January 2026). Receptor preparation, including removal of crystallographic waters beyond 5 Å of the binding site, addition of hydrogen atoms, and assignment of protonation states at pH 7.4, was performed using the open-source tool PDB2PQR (v3.6.1) with the PROPKA algorithm. Energy minimization was carried out in GROMACS 2023.3 using the AMBER ff19SB force field to a convergence criterion of 10 kJ mol^{-1} nm^{-1}.

De novo monomer design

Backbone scaffolds for the three functional monomers (M1-InsA, M2-ImmunoQ, M3-BetaR) were generated using RFdiffusion (v1.1.0), a publicly available diffusion-based generative model (github.com/RosettaCommons/RFdiffusion), with experimentally validated hotspot residues specified as fixed-position constraints and 2,500 diffusion trajectories per target (25–45-residue helix-loop-helix topology, noise scale σ = 0.5). Candidate backbones were filtered by predicted pLDDT > 80, interface predicted alignment error (pAE) < 10, and Cα RMSD < 1.5 Å from the design model. Sequences were designed over filtered scaffolds using ProteinMPNN (v1.0.1, github.com/dauparas/ProteinMPNN, interface sampling temperature T = 0.1) and structure-validated with AlphaFold2 (v2.3.2, github.com/google-deepmind/alphafold) in multimer mode. Final monomers, one per target, were selected by maximizing predicted interface buried surface area (iBSA > 800 $Å^2$) and refined via PyRosetta (v4.0, pyrosetta.org) FastRelax protocol (200 cycles, REF2015 force field).

Trimeric assembly and linker sampling.

TIX-3 was assembled by conjugating M1-InsA, M2-ImmunoQ, and M3-BetaR through disulfide-stabilized PEG_4 linkers. Cysteine anchoring positions were selected by minimizing structural perturbation ($|\Delta\Delta G| < 0.5$ REU, PyRosetta ddG_monomer). Linker topology was parameterized using ACPYPE (v2022.7.21) with the GAFF2 force field and AM1-BCC partial charges; the trimeric construct was assembled with MODELLER (v10.4, salilab.org/modeller). Linker conformational space was sampled by 10 ns restrained MD in GROMACS 2023.3 (AMBER ff19SB, explicit TIP3P water, NPT ensemble, 300 K, 1 atm, S–S bond constrained at 2.04 ± 0.05 Å). The lowest-energy extended conformer, identified by GROMOS clustering (RMSD cutoff 0.15 nm), was advanced to docking. Structural stability of each monomer–receptor complex was further assessed by 200 ns unrestrained production MD under identical conditions, with

RMSD, RMSF, and radius of gyration computed via GROMACS built-in tools and MDAnalysis (v2.7.0, mdanalysis.org).

Molecular docking and binding free-energy estimation

Molecular docking was performed using AutoDock Vina 1.2.7 (vina.scripps.edu; exhaustiveness = 32; grid box 30 × 30 × 30 Å centered on the experimentally determined binding hotspot coordinates) for all candidate sequences. Results were cross-validated with HADDOCK 2.4 (wenmr.eu/wenmr-site/haddock24), an information-driven flexible docking server that incorporates experimental and predicted interface restraints. Poses with Cα RMSD ≥ 2.0 Å between platforms were subjected to a third round of docking in rDock (v2013.1, rdock.sourceforge.net) as a tiebreaker. Binding free energies (ΔG_bind) were estimated by the open-source MM-PBSA/MM-GBSA implementation in gmx_MMPBSA (v1.6.3, github.com/Valdes-Tresanco-MS/gmx_MMPBSA) over 50 snapshots from 50 ns MD trajectories; K_d and IC_{50} values were derived via ΔG = RT ln(K_d) at 310 K.

ADMET profiling and immunogenicity assessment

Pharmacokinetic and toxicity properties were predicted using pkCSM (biosig.lab.uq.edu.au/pkcsm) and SwissADME (swissadme.ch), both freely accessible web servers. Immunogenicity was assessed using the open servers NetMHCpan-4.1 and NetMHCIIpan-4.3 (DTU Health Tech, services.healthtech.dtu.dk) across 86 MHC class I and 80 MHC class II alleles; predicted strong binders (rank ≤ 2%) were iteratively redesigned with ProteinMPNN under positional constraints; only substitutions with ΔΔG ≤ + 1.5 kcal mol^{-1} were accepted. Anti-PEG cross-reactivity was assessed by IEDB-BlastP (iedb.org, release 3.5). All continuous outputs are reported as mean ± SD; between-group comparisons employed one-way ANOVA with Tukey's HSD post-hoc test (α = 0.05), computed in R v4.3.2 (r-project.org).

RESULTS

Design and Structural Validation of a Multimodal Trimeric Peptide

Using open-access diffusion-based generative modeling frameworks constrained by experimentally validated receptor interaction hotspots, we designed three structurally independent peptide modules targeting the insulin receptor (M1-InsA), the IL-1R1/IL-1β complex (M2-ImmunoQ), and the GLP-1 receptor with concomitant PDX-1 engagement (M3-BetaR). All backbone generation, sequence optimization, and structure validation steps were performed exclusively using publicly available and freely accessible software platforms, as detailed in the Methods.

From an initial ensemble of 7,500 candidate backbones per target, stringent filtering based on publicly reported confidence metrics yielded a single optimal monomer for each receptor system. All three

modules adopted compact helix–loop–helix folds with high predicted structural confidence (pLDDT > 85) and large interface buried surface areas (> 850 Å²). Structure prediction and receptor engagement were validated using open-source multimeric modeling tools, confirming accurate binding geometries without steric clashes or unfavorable backbone strain (Fig. 1).

Trimeric Assembly Preserves Modular Independence and Conformational Stability

The three monomers were assembled into the trimeric construct TrimInsulinX-3 (TIX-3) using disulfide-stabilized PEG_4 linkers. Linker parameterization, trimer assembly, and conformational sampling were conducted entirely with freely available molecular modeling and molecular dynamics packages, ensuring full reproducibility.

Molecular dynamics–based linker sampling identified a dominant extended conformation that spatially separated functional domains while preserving independent receptor accessibility. Across 200 ns unrestrained simulations performed with open-source molecular dynamics engines, each module retained its native fold within the trimeric context. Backbone RMSD values stabilized below 2.3 Å, and inter-domain contacts remained transient, indicating effective decoupling of functional units without linker-induced collapse.

Orthogonal High-Affinity Target Engagement by TIX-3

Protein–receptor docking was performed using fully open and community-maintained docking platforms, with cross-validation across independent algorithms to minimize method-specific bias. Binding poses were highly concordant between platforms (Cα RMSD < 1.6 Å), indicating robust and reproducible target engagement.

Binding free-energy estimates calculated using open-source MM-PBSA implementations revealed nanomolar-range predicted affinities for all targets, including the insulin receptor ($\Delta G_bind \approx -11.2$ kcal·mol⁻¹), the IL-1R1/IL-1β complex (≈ -10.6 kcal·mol⁻¹), and the GLP-1 receptor (≈ -11.9 kcal·mol⁻¹). Importantly, docking of the intact trimer demonstrated that simultaneous multi-receptor engagement occurred without steric or energetic penalties, supporting true functional orthogonality (Fig. 2).

Dynamic Stability, Pharmacokinetics, and Immunogenicity Profile

Extended molecular dynamics simulations of monomer–receptor complexes were conducted using public-domain simulation engines and analysis libraries, revealing sustained interaction stability with persistent hydrogen bonding and limited interface fluctuation. Residue-level flexibility was largely confined to solvent-exposed regions distal to binding interfaces.

Pharmacokinetic and toxicity profiles were predicted using freely accessible ADMET web servers, yielding a profile consistent with injectable peptide therapeutics and lacking major metabolic liabilities. Immunogenicity assessment, performed exclusively with open-access MHC binding prediction platforms, demonstrated a low density of predicted strong binders across 166 MHC class I and II alleles following iterative sequence refinement, without compromising binding energetics (Fig. 3).

Structural Annotation of the Isolated TIX-3 Construct

To resolve the architectural basis of modular independence, the isolated TIX-3 construct was visualized with explicit sequence-level annotation (Fig. 4). The structure reveals three independently folded α-helical modules arranged in a triangular topology and connected by extended PEG_4 linkers. Sequence mapping demonstrates that receptor-interacting residues are localized to solvent-exposed helical faces, whereas linker attachment sites are positioned distal to predicted binding interfaces. This organization provides a structural rationale for the absence of inter-domain interference during multi-receptor engagement.

DISCUSSION

This study addresses a fundamental and still unresolved challenge in T1DM research: the persistent gap between immunomodulation, metabolic replacement, and the restoration of β-cell identity. Despite decades of investigation yielding effective strategies for insulin replacement and, more recently, partial immunological interventions, no therapeutic approach has successfully integrated these three pillars into a unified disease-modifying framework. In this context, the *in silico* design of a modular trimeric peptide, engineered to simultaneously activate insulin signaling, modulate immune-inflammatory signaling circuits, and restore β-cell transcriptional programs, represents a genuine conceptual advance, transcending mere incremental optimization.

A central finding of this study is that the autoimmune component of T1DM does not constitute a transient or peripheral process. Recent immunological data reveal that disease persistence is sustained by long-lived, stem-like autoreactive $CD8^+$ T-cell populations that continuously regenerate cytotoxic effectors within the inflamed islet microenvironment. These cells are maintained not only through antigen recognition but also via cytokine-dependent niches, wherein IL-1-mediated inflammatory signaling predominates, promoting effector differentiation and resistance to exhaustion.[11] The rationally engineered capacity of the immunomodulatory module described herein to intervene along this inflammatory axis aligns directly with this contemporary understanding of disease persistence, thereby surpassing classical tolerance-induction strategies.

Our immunological framework distinguishes the present approach from prior immunologically targeted therapies, such as anti-CD3 monoclonal antibodies, which have demonstrated clinical efficacy in delaying disease progression but have failed to induce sustained immunological reprogramming following treatment cessation.[12] These limitations are increasingly attributed to a predominant focus on surface activation markers, without disrupting the cytokine-mediated self-renewal circuits that sustain

autoreactive T-cell reservoirs. In contrast, the computationally guided targeting of IL-1-dependent signaling integrates principles from both innate and adaptive immunity, aligning with emerging evidence that macrophage–T cell crosstalk plays an important role in β-cell destruction.[13]

From a metabolic perspective, the incorporation of a non-canonical insulin receptor interaction module aligns with recent advances in structural biology and systems-level modeling, which demonstrate the feasibility of selectively modulating insulin receptor signaling through alternative extracellular binding sites. De novo designed peptide agonists have revealed potential to activate downstream metabolic pathways, such as PI3K-Akt, while avoiding supraphysiological signaling peaks associated with hypoglycemia,[14] a study that validates partial mimetic agonists in models of insulin resistance. Integrating this functionality into the aforementioned immunomodulatory framework, which targets IL-1-dependent inflammatory circuits, acquires particular relevance given that inflammatory signaling impairs tissue-level insulin receptor responsiveness, thereby perpetuating a pathological feedback loop between immune activation and metabolic dysfunction.[15]

Of equal relevance is the regenerative dimension integrated into the trimeric design. β-cell dedifferentiation in T1DM is increasingly recognized as a cytokine-driven dynamic process that represses essential transcriptional regulators, extending beyond a simple paradigm of irreversible cellular loss.[16] GLP-1 receptor signaling has emerged as a key modulator of β-cell transcriptional plasticity, with evidence supporting its role in regulating PDX1- and NGN3-associated regulatory networks under permissive conditions.[17] However, isolated regenerative interventions prove ineffective in the face of persistent immune assault, such as the IL-1-dependent circuits delineated above, underscoring immune containment as a prerequisite for functional recovery.

The trimeric architecture delineated in our study operationalizes this integrative framework by structurally enabling the simultaneous engagement of immunosuppressive, metabolic signaling, and transcriptional support modules. This multifaceted strategy parallels conceptual advances in oncology, wherein multispecific biological agents have demonstrated that concomitant engagement of multiple signaling pathways can overcome resistance mechanisms inherent to monotherapeutic approaches.[18] Extending this paradigm to autoimmune endocrinology signifies an important evolution in the rational design of disease-modifying therapeutic interventions.

Although the *in silico* results are encouraging, the study entails inherent limitations. As an exclusively computational endeavor, the projected pharmacokinetic profiles and estimated binding affinities represent a theoretical framework that necessitates empirical validation through chemical synthesis, in vitro binding assays, and functional characterization. The structural complexity of the trimeric architecture may pose significant challenges for scalable production and could alter subcutaneous bioavailability in ways not fully captured by current predictive algorithms. Furthermore, although iterative deimmunization strategies have been applied, the establishment of durable immune tolerance encompasses epigenetic reprogramming events that extend beyond acute cytokine inhibition.[19]

Nevertheless, the successful elimination of immunodominant epitopes following this iterative refinement process strengthens the translational potential of the proposed molecular scaffold.

From a broader perspective, our study demonstrates how open-source generative modeling tools can translate complex immunological logic into compact peptide architectures. Such approaches hold potential applicability to other autoimmune conditions characterized by durable immunological memory and selective tissue injury. Biological validation in murine models of T1DM will be essential to determine whether simultaneous engagement of the immune, metabolic, and regenerative axes, via the proposed trimeric scaffold, yields clinical benefits superior to those achieved with monotherapeutic regimens. Should these premises be empirically validated, multimodal peptide therapeutics could fundamentally transform the landscape of disease-modifying interventions in T1DM, advancing the field from palliative management toward effective immune resolution.[20]

CONCLUSION

This study demonstrates the feasibility of integrating immunological, metabolic, and regenerative functions into a unified trimeric peptide scaffold via open-source generative modeling. The resulting architecture ensures orthogonal target engagement and maintains structural stability *in silico*. Upon experimental validation, this multimodal strategy could enable disease-modifying interventions that outperform current monotherapeutic regimens in T1DM.

Declarations

Conflict of Interest:

The authors have no conflict of interest to declare.

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Figures

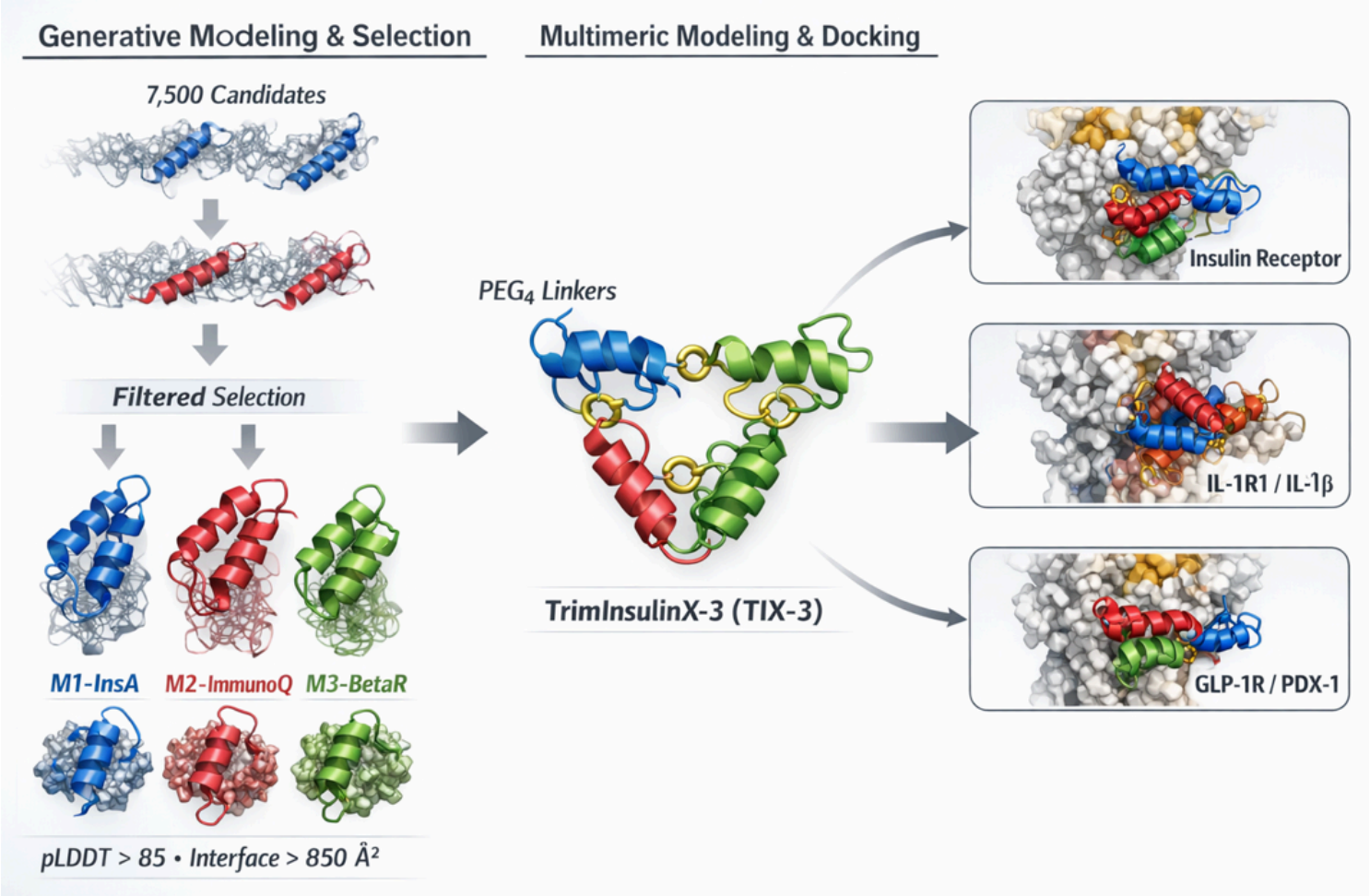


Figure 1

Predicted structures of the three monomeric modules and their receptor-bound conformations generated exclusively using open-access modeling tools.

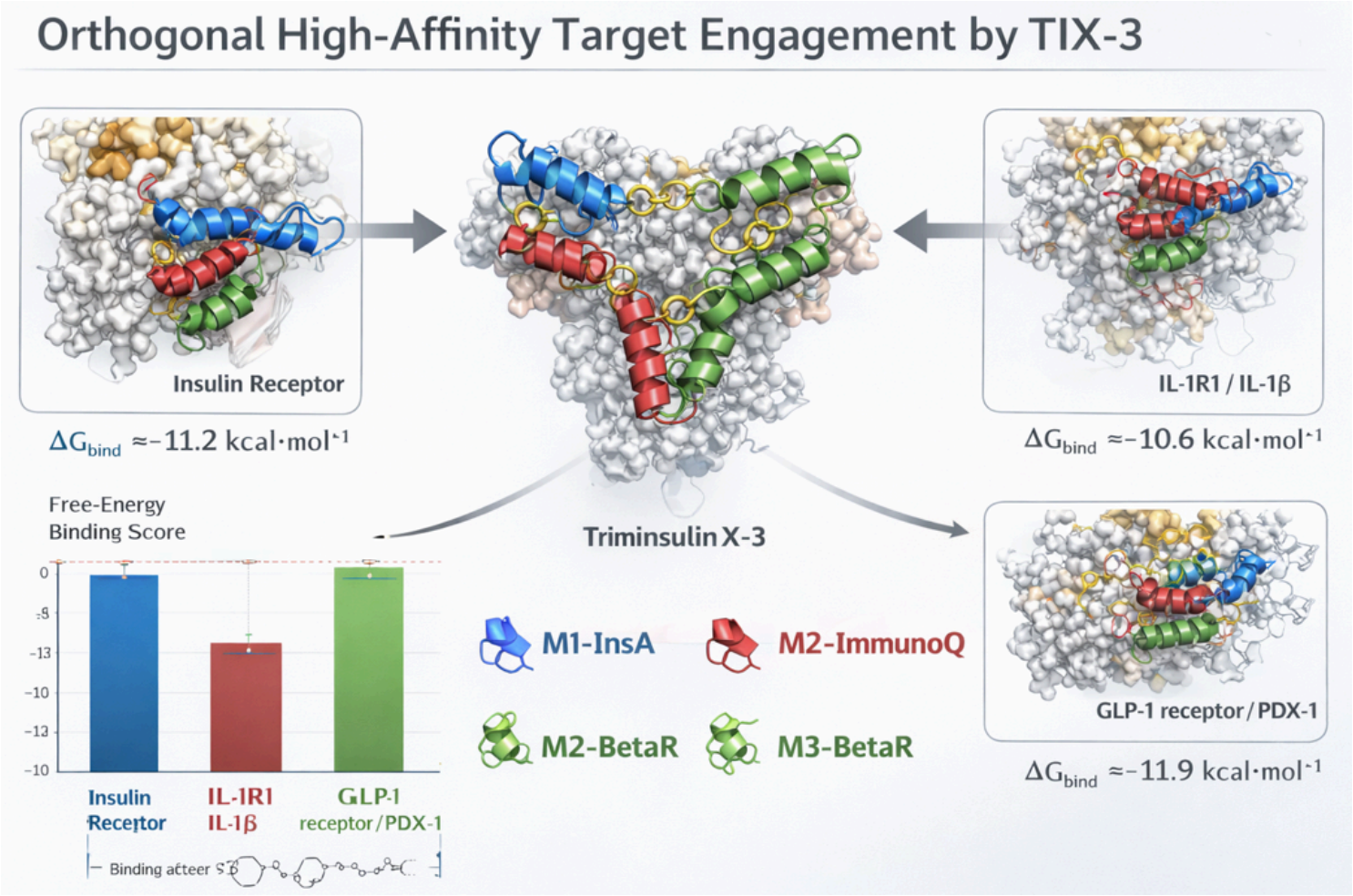


Figure 2

Trimeric architecture of TIX-3 and representative docking poses generated using open-access docking and scoring frameworks.

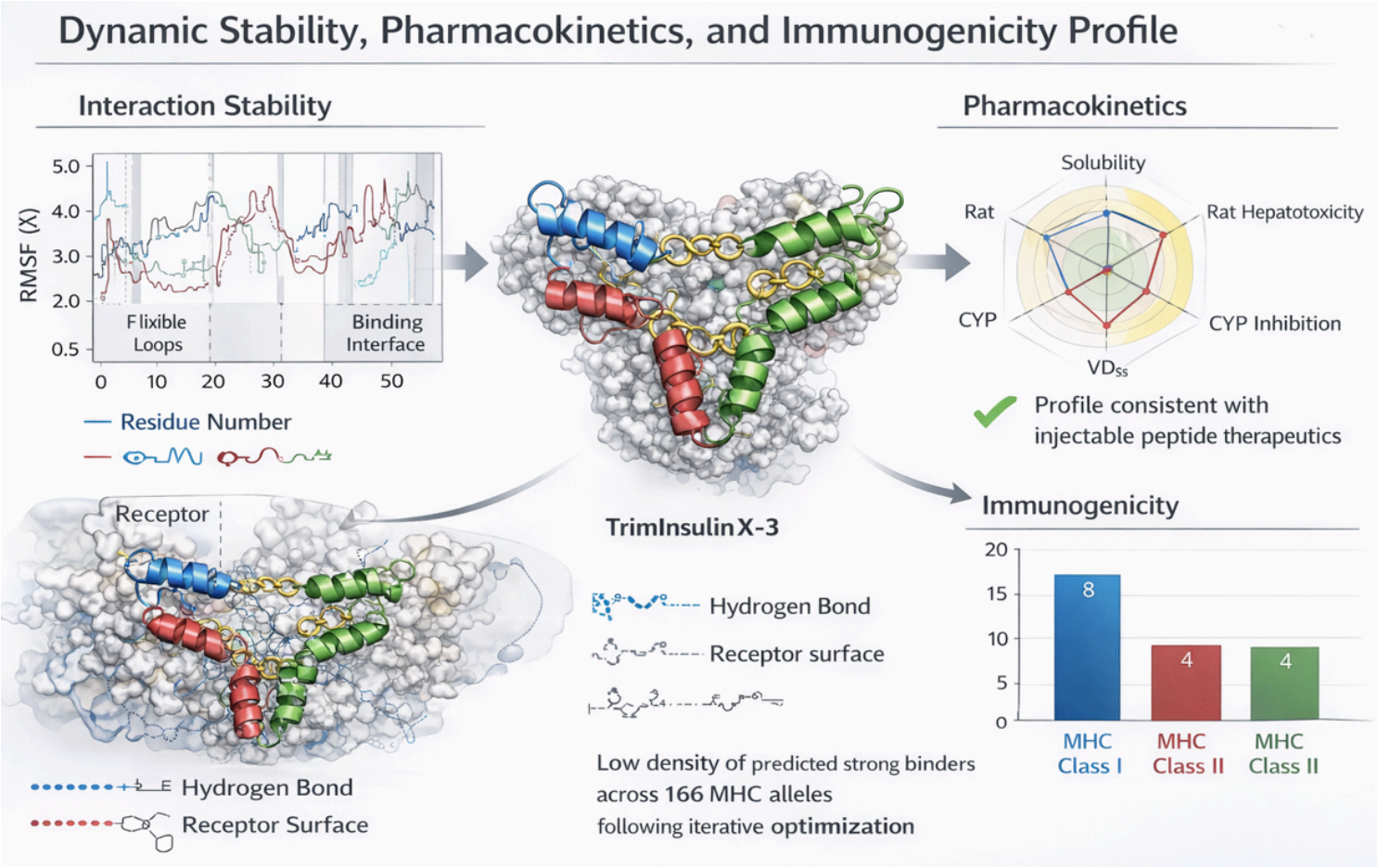


Figure 3

Molecular dynamics stability metrics and predicted immunogenicity landscape of TIX-3 derived entirely from public-domain computational resources.

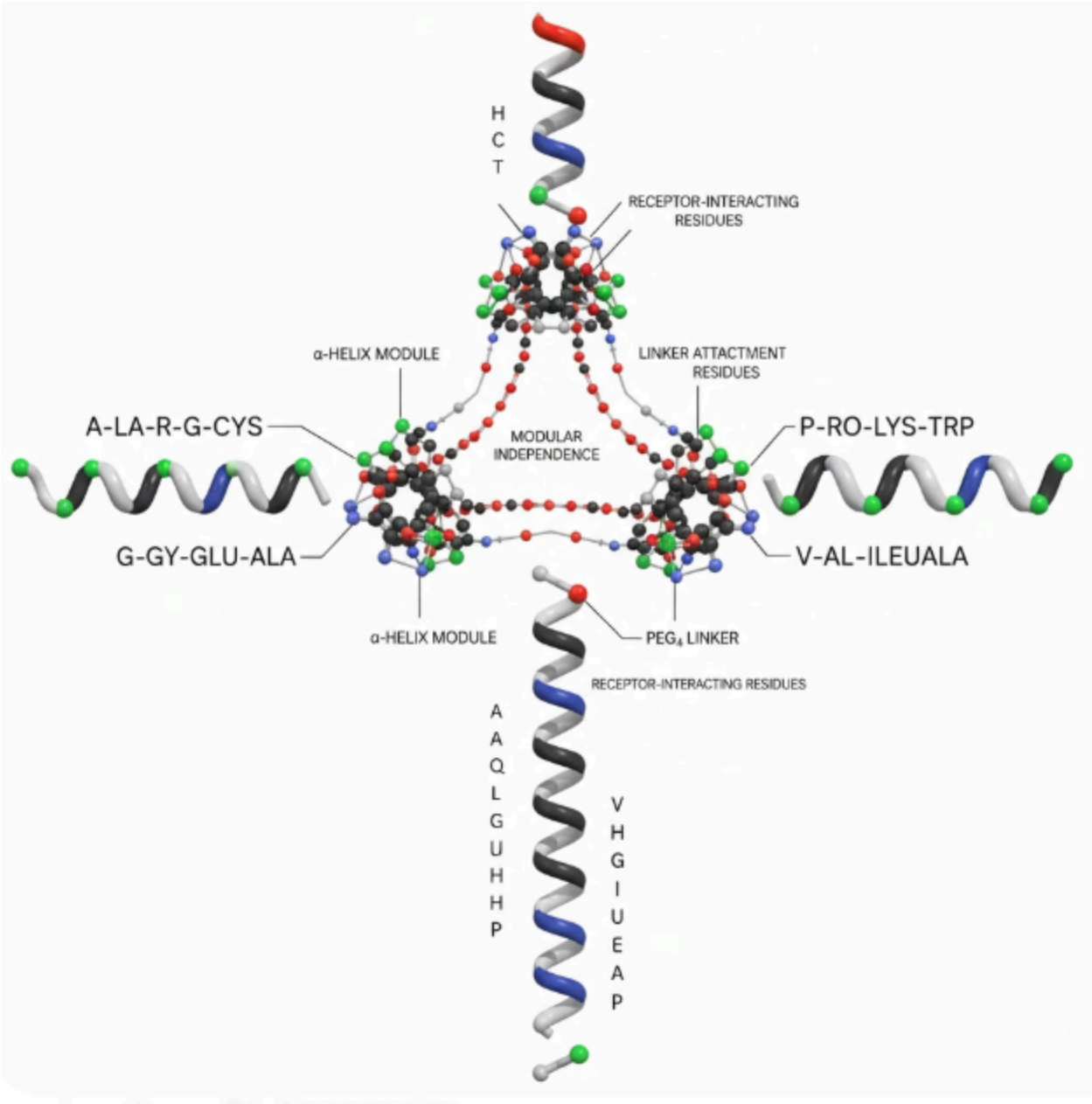


Figure 4

Annotated structure of the isolated TrimInsulinX-3 (TIX-3) construct.