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Decision-Gated In Silico Framework and Prototype Chemotype for Molecular Glue Stabilization of the PDX1–MAFA–NKX6.1 Complex in Type 2 Diabetes

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Short Report

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Abstract

Introduction/Aims: Loss of mature pancreatic β-cell identity under chronic glucolipotoxicity and oxidative stress drives progressive insulin secretory failure in type 2 diabetes. Mature identity depends on cooperative nuclear activity of PDX1, MAFA, and NKX6.1, which undergo cytoplasmic mislocalization and turnover under metabolic stress. Because transcription factors lack deep catalytic pockets, no drug class stabilizes this assembly. We aimed to establish a decision-gated computational framework to nominate small-molecule stabilizers (molecular glues) of the PDX1–MAFA–NKX6.1 complex.

Methods: We integrated human islet pathology, murine lineage-tracing evidence, and structural bioinformatics into a five-stage, gate-controlled pipeline targeting human PDX1 (P52945), MAFA (Q8NHW3), and NKX6.1 (P78426). The cascade comprised: (1) multimer modeling and explicit-solvent molecular dynamics benchmarked against scrambled-interface controls (Gate G1); (2) consensus pocket detection (Gate G2); (3) hierarchical virtual screening with free-energy and cooperativity rescoring, ΔΔG_coop (Gate G3); and (4) physicochemical, PAINS, and homeodomain/bZIP selectivity filtering (Gate G4), culminating in prospective biophysical and human β-cell assays (Gate G5). Using RDKit, we generated a reference chemotype to parameterize pharmacophoric boundaries.

Results: Evidence grading confirmed strong human (Tier 1) and genetic (Tier 2) validation for the triad but absent druggability evidence (Tier 3). The protocol defined preregistered stopping rules and three falsifiable predictions paired with SPR/ITC, NanoBRET, and GSIS assays, anchored by a PAINS-free prototype scaffold (C22H25N5O; MW 375.48 Da; cLogP 2.97; QED 0.745).

Conclusions: This framework converts β-cell identity preservation into an auditable, falsifiable discovery pipeline enforcing prescreening model deposition and cooperativity gates before nomination.

Research in Context

What is already known about this subject?

• Progressive β-cell failure in type 2 diabetes involves stress-induced dedifferentiation and loss of mature endocrine identity rather than β-cell apoptosis alone.
• The transcription factors PDX1, MAFA, and NKX6.1 cooperate to maintain mature β-cell identity and insulin secretory competence, but undergo selective depletion and nuclear exclusion under chronic oxidative and glucolipotoxic stress.
• Molecular glues can cooperatively stabilize shallow, composite protein–protein interfaces that are intractable to classical orthosteric ligands.

What is the key question?

• How can the hypothesis of pharmacologically stabilizing the PDX1–MAFA–NKX6.1 transcriptional complex be translated into a rigorous, falsifiable, decision-gated computational discovery pipeline?

What are the new findings?

• We define a five-stage, gate-controlled computational and translational framework (Gates G1–G5) incorporating scrambled-interface negative controls, multi-method consensus pocket detection, cooperativity-weighted free-energy rescoring (ΔΔG_coop), and paralog selectivity filtering.

• We establish three quantitative, experimentally falsifiable predictions linking biophysical stabilization to cellular proximity (NanoBRET) and functional glucose-stimulated insulin secretion rescue, anchored by an RDKit-validated open-source prototype reference chemotype.

How might this affect clinical practice in the foreseeable future?

• Nominating selective stabilizers of the PDX1–MAFA–NKX6.1 triad could establish a first-in-class, disease-modifying therapy that preserves endogenous β-cell identity and sustains responsiveness to existing incretin or secretagogue therapies.

1. INTRODUCTION

Type 2 diabetes arises from peripheral insulin resistance combined with progressive β-cell secretory failure. Lineage tracing showed that β cells under sustained metabolic stress can lose mature identity and acquire progenitor-like features without dying,[1] and islets from donors with type 2 diabetes contain hormone-depleted endocrine cells with features of dedifferentiation.[2]

Mature identity depends on a restricted network of transcription factors. Oxidative stress drives cytoplasmic translocation of MAFA and NKX6.1 and impairs PDX1 promoter occupancy, and all three factors are selectively reduced in human type 2 diabetes islets.[3] Conditional deletion studies established that PDX1, MAFA, and NKX6.1 each make a nonredundant contribution to identity and secretory function.[4–6]

No glucose-lowering drug class directly stabilizes this assembly. Transcription factors lack deep catalytic pockets and act through extended protein–protein and protein–DNA interfaces, which makes them difficult targets for occupancy-driven pharmacology.[7] Molecular glues, small molecules that bind cooperatively at composite interfaces to stabilize native protein–protein interactions, offer a modality suited to this problem,[8] and deep-learning multimer prediction now permits structural hypotheses for such assemblies to be generated in silico.[9] However, a decision-gated pipeline that converts the PDX1–MAFA–NKX6.1 stabilization hypothesis into an auditable workflow with prespecified stopping rules has not been described.

Here we define such a framework. It integrates multimer modeling, orthogonal consensus pocket detection, hierarchical virtual screening with cooperativity rescoring, and selectivity filtering, with each stage closed by an explicit quantitative gate. We also state three falsifiable predictions and generate an

open-source prototype chemotype in RDKit to benchmark physicochemical boundaries. The framework is methodological and reports no biological activity.

2. METHODS

2.1 Design principles and preregistration governance

The framework was designed as a hypothesis-generating protocol built on three operational principles: (i) every computational stage ended in a prespecified quantitative decision gate (G1–G4); (ii) ligand-based virtual screening began only after structural models of the target interface had been generated, evaluated against scrambled negative controls, and publicly deposited; and (iii) the computational pipeline terminated at candidate nomination for experimental triage (Stage 5). The protocol required that all algorithmic hyperparameters, scoring cutoffs, and negative-control definitions be recorded in a time-stamped analysis plan before computation to prevent post hoc threshold adjustment.

2.2 Target definition and three-tier evidence grading

The molecular targets were human PDX1 (UniProt P52945; 283 residues, homeodomain 146–206), human MAFA (Q8NHW3; 353 residues, basic leucine-zipper [bZIP] domain 230–295), and human NKX6.1 (P78426; 367 residues, homeodomain 306–366). Both full-length sequences and domain-truncated constructs encompassing structured DNA-binding and transactivation-flanking segments were specified to control conformational noise from intrinsically disordered regions. Prior evidence was graded into three formal tiers (Table 1): human islet pathology, mouse genetic and lineage-tracing data, and direct structural or pharmacological druggability evidence.

Table 1
Three-tier translational and structural evidence hierarchy for the PDX1–MAFA–NKX6.1 transcriptional complex

Tier	Evidence Domain	Empirical Basis & Literature Support	Status & Translational Implication
Tier 1	Human pancreatic islet observations	Quantitative immunohistochemistry and islet profiling showing selective depletion and cytoplasmic mislocalization of PDX1, MAFA, and NKX6.1 in type 2 diabetes[2,3]	Strong: direct clinical association with β-cell dedifferentiation in human disease
Tier 2	Mouse genetic and lineage-tracing data	Conditional β-cell knockouts demonstrating nonredundant requirement of each factor[4–6]; oxidative stress inactivates the triad and antioxidant rescue restores nuclear localization[1,3]	Strong: causal validation that preserving nuclear complex activity maintains β-cell identity
Tier 3	Direct structural or pharmacological druggability	Experimental cryo-EM/X-ray ternary complexes and small-molecule stabilizers of the PDX1–MAFA–NKX6.1 interface	Absent: no experimental ternary structure or validated small-molecule glue reported

2.3 Five-stage gated architecture

The framework comprised four sequential computational stages (Stages 1–4) and one prospective experimental validation stage (Stage 5), closed by explicit decision gates (Fig. 1, Table 2).

Stage 1: Multimer structural modeling and negative-control benchmarking (Gate G1).

The protocol specified modeling of binary (PDX1–MAFA) and ternary (PDX1–MAFA–NKX6.1) assemblies with deep-learning multimer prediction methods (AlphaFold-Multimer and AlphaFold 3 implemented through ColabFold with MMseqs2 alignments). It also specified sampling of alternative interface geometries through multi-seed variation and domain-mapping constraints. Top-ranked models, selected by interface predicted TM-score (ipTM) and predicted aligned error (PAE), were designated for explicit-solvent molecular dynamics (MD) refinement with the AMBER ff14SB force field in GROMACS. Scrambled-interface and charge-reversal mutant complexes were designated for parallel simulation as negative controls. Gate G1 required statistically superior interface contact persistence and lower interface RMSD relative to scrambled controls ($P < 0.01$) before public repository deposition.

Stage 2: Orthogonal consensus pocket detection (Gate G2).

The protocol specified detection of composite interface and allosteric cavities across MD-refined ensembles with three orthogonal algorithms: geometric alpha-sphere analysis (Fpocket), grid-based druggability scoring (DoGSiteScorer), and solvent-mapping hot-spot identification (FTMap). Cavities present in isolated monomers were subtracted to isolate interface-dependent pockets. Gate G2 required

recovery by ≥ 2 independent algorithms, a pocket volume ≥ 150 Å³, and evolutionary conservation of pocket-lining residues.

Stage 3: Hierarchical virtual screening and cooperativity rescoring (Gate G3).

The protocol specified docking of fragment-like and lead-like libraries into consensus interface pockets with AutoDock Vina 1.2, rescoring of top poses by end-point molecular mechanics generalized Born surface area (MM/GBSA) calculations in gmx_MMPBSA, and expansion of chemical diversity through pharmacophore and three-dimensional shape-based searches. Molecular glue behavior was defined by a thermodynamic cooperativity metric that compared complex and monomer binding free energies: $\Delta\Delta G_{coop} = \Delta G_{complex} - \min(\Delta G_{PDX1}, \Delta G_{MAFA})$. Gate G3 required a post-docking MD ligand RMSD < 2.0 Å and $\Delta\Delta G_{coop} \leq -2.0$ kcal/mol.

Stage 4: Physicochemical, PAINS, and off-target selectivity filtering (Gate G4).

The protocol specified evaluation of candidates that passed Gate G3 for drug-like physicochemical properties (Lipinski and Veber compliance and Quantitative Estimate of Drug-likeness [QED]) with SwissADME and RDKit, followed by filtering against pan-assay interference (PAINS) substructures. Selectivity assessment comprised counter-docking against homologous homeodomain proteins (NKX2.2, PAX6, ARX) and bZIP paralogs (MAFB, c-JUN), together with sequence-divergence analysis of pocket-lining residues. Gate G4 required zero PAINS alerts, QED ≥ 0.60, and an off-target binding free-energy selectivity margin ≥ 1.5 kcal/mol.

Stage 5: Prospective experimental cascade (Gate G5).

Shortlisted candidates were designated for direct biophysical binding and thermal stabilization assays (SPR, ITC, or nanoDSF), live-cell NanoBRET proximity assays under basal and glucolipotoxic conditions, and functional rescue assays (glucose-stimulated insulin secretion [GSIS] and maturity transcript profiling) in human β-cell models. This stage was outside the computational pipeline.

Table 2
Stage architecture, principal analytical methods, primary outputs, and quantitative decision gates

Stage	Principal Methods	Primary Output	Quantitative Decision Gate
1 (In silico)	Multimer prediction; explicit-solvent MD; scrambled-interface negative controls	Ranked binary/ternary structural ensembles deposited publicly	G1: Interface contact persistence exceeds scrambled controls ($P < 0.01$)
2 (In silico)	Multimethod pocket detection: Fpocket, DoGSiteScorer, FTMap; monomer subtraction	Prioritized consensus composite pockets	G2: Pocket supported by ≥ 2 methods; volume ≥ 150 Å³; conserved lining residues
3 (In silico)	Docking (AutoDock Vina 1.2); gmx_MMPBSA rescoring; ΔΔG_coop metric	Cooperativity-ranked candidate library	G3: Stable pose (RMSD < 2.0 Å) + cooperativity gain ΔΔG_coop ≤ − 2.0 kcal/mol
4 (In silico)	Property filters (SwissADME/RDKit); PAINS removal; paralog counter-docking	Drug-like, selective candidate shortlist	G4: 0 PAINS alerts; QED ≥ 0.60; off-target selectivity margin ≥ 1.5 kcal/mol
5 (Prospective)	SPR/ITC/nanoDSF; live-cell NanoBRET; human β-cell GSIS & transcriptomics	Biophysical and cellular proof-of-mechanism	G5: Direct Kd/ΔTm shift + nuclear stabilization and GSIS rescue under stress

2.4 Decision gates, orthogonal controls, and open-source prototype parameterization

Decision gates required convergence across orthogonal methods rather than reliance on single-score heuristics. Embedded controls comprised scrambled-interface decoys (Stage 1), monomer-versus-complex pocket subtraction (Stage 2), redocking validation and property-matched decoys (Stage 3), and inclusion of native PDX1–MAFA interfaces as internal references during counter-docking (Stage 4). To establish a concrete physicochemical and pharmacophoric reference standard for Stage 3–4 virtual screening filters without claiming biological activity, a prototype small-molecule stabilizer chemotype was designed and energy-minimized with the open-source cheminformatics toolkit (RDKit version 2026.09.2; Python 3.15.2). Three-dimensional conformers were embedded with the Experimental-Torsion Basic Knowledge Distance Geometry algorithm (ETKDGv3) and optimized with the Merck Molecular Force Field (MMFF94s), and substructure hygiene was verified against the Baell–Holloway PAINS catalog.

3. RESULTS

3.1 Evidence hierarchy and translational gap

Tier 1 and Tier 2 evidence robustly establish the biological necessity of the PDX1–MAFA–NKX6.1 transcriptional triad in maintaining mature human and murine β-cell identity (Table 1). Conversely, Tier 3 evidence is entirely absent: no small-molecule stabilizer of the interface and no experimental cryo-EM or X-ray structure of the ternary assembly have been reported. The complex is therefore validated as a high-priority disease-relevant node but remains untested as a direct pharmacological target.

3.2 Framework deliverables and experimentally falsifiable predictions

The framework delivers a five-stage gate-controlled workflow (Table 2), a formal three-tier evidence hierarchy (Table 1), a conceptual mechanistic model of stress-induced destabilization and glue-mediated rescue (Fig. 2), and three sequentially falsifiable experimental predictions paired with quantitative rejection criteria (Table 3).

Table 3
Preregistered testable predictions, primary experimental assays, and quantitative falsification criteria

Prediction	Mechanistic Hypothesis	Primary Assay	Quantitative Falsification Criterion (No-Go)
P1	Nominated candidate increases PDX1–MAFA thermodynamic stability and apparent affinity	Recombinant SPR, ITC, or nanoDSF (± candidate ligand)	No statistically significant shift in melting temperature (ΔTm < 1.0°C) or Kd vs. DMSO vehicle
P2	Candidate stabilizes nuclear PDX1–MAFA–NKX6.1 proximity in live β cells under metabolic stress	Live-cell NanoBRET under basal vs. glucolipotoxic conditions (palmitate + high glucose)	No significant attenuation of stress-induced NanoBRET proximity loss vs. vehicle control
P3	Complex stabilization preserves mature β-cell identity transcripts and secretory competence	RT-qPCR/RNA-seq (INS, MAFA, NKX6.1, ALDH1A3) and GSIS in human β-cell models	Maturity transcripts and GSIS decline, and ALDH1A3 rises, indistinguishably from vehicle

3.3 Cheminformatic characterization of the open-source prototype glue chemotype

Using RDKit, we generated an sp3-enriched, drug-like reference molecular glue prototype designed to engage composite polar–hydrophobic grooves characteristic of homeodomain–bZIP protein–protein interfaces (Fig. 3). The canonical SMILES string is Cc1cc(C(= O)NC2CCN(Cc3ccncc3)CC2)ccc1-n1cccn1 (IUPAC: N-[1-(pyridin-4-ylmethyl)piperidin-4-yl]-3-methyl-4-(1H-pyrazol-1-yl)benzamide; molecular formula: C22H25N5O; InChIKey: SDOAWXOBKIIXBG-UHFFFAOYSA-N).

Multiparameter physicochemical auditing confirmed full compliance with Gate G4 medicinal chemistry filters: molecular weight = 375.48 Da (< 500 Da), calculated lipophilicity cLogP = 2.97 (optimal window 1.5–3.5), topological polar surface area TPSA = 63.05 Å² (favorable for cell-membrane and nuclear-

envelope permeability), 1 hydrogen-bond donor, 5 hydrogen-bond acceptors, 5 rotatable bonds, a Quantitative Estimate of Drug-likeness (QED) of 0.745 (threshold ≥ 0.60), and 0 PAINS structural alerts. Structurally, the central secondary carboxamide provides a directional hydrogen-bond donor/acceptor bridge across backbone interface atoms, flanked by a basic 4-pyridinylmethyl-piperidine moiety capable of forming electrostatic interactions with conserved acidic patches on the homeodomain surface and a 3-methyl-4-(1H-pyrazol-1-yl)phenyl group tailored for shallow hydrophobic subpockets.

3.4 Risk register and embedded gate mitigations

Key technical risks include inaccurate multimer prediction for flexible transcription factor interfaces, incomplete conformational sampling of stress-modified states, limited ranking accuracy of static docking scores, low baseline frequency of true cooperative stabilizers, and potential off-target activity in non-β tissues expressing PDX1 (such as duodenal epithelium and exocrine pancreas). Each risk is paired with an explicit mitigation embedded in gate logic: domain-truncated ensemble modeling and scrambled negative controls (G1), multi-method consensus pocket detection with monomer subtraction (G2), MM/GBSA rescoring and ΔΔG_coop filtering (G3), pharmacophore/shape expansion around cooperative hits, and paralog counter-docking plus sequence-divergence mapping (G4).

4. DISCUSSION

This study shows that stabilization of the PDX1–MAFA–NKX6.1 complex, a node that sustains mature β-cell identity, can be approached through an auditable and falsifiable computational path. The evidence grading identified strong human and genetic support but no druggability evidence, which defines the translational gap. The framework addresses that gap with prespecified decision gates and three testable predictions, and the prototype chemotype met the physicochemical filters. If confirmed experimentally, the approach could shift antidiabetic drug discovery from stimulating or replacing β cells toward preserving their identity.

The framework addresses a therapeutic gap that earlier work on β-cell dedifferentiation left open. Recent reviews describe reversal of identity loss as a realistic treatment aim in type 2 diabetes,[10,11] and preclinical studies support that view. Insulin therapy allowed dedifferentiated β cells to regain a mature insulin-positive phenotype in diabetic mice,[12] a TGF-β receptor I inhibitor restored mature identity even after prolonged diabetes,[13] and β-cell-targeted delivery of estrogen through a GLP-1 conjugate further increased β-cell survival and regeneration.[14] Each of these interventions acts through glycemic load, upstream signaling, or hormonal pathways, and, to our knowledge, none engages the transcription factor assembly itself, which is consistent with the absence of Tier 3 evidence in the present grading. A stabilizer would therefore complement these strategies instead of duplicating them.

The readouts chosen for prediction P3 are consistent with the same literature. ALDH1A3 marks a subset of failing β cells in diabetic mice,[15] and its genetic ablation or pharmacological inhibition has been proposed as a strategy against β-cell failure,[16] which supports its use as a stress-sensitive transcript

alongside the maturity genes. The prediction that stabilization should blunt ALDH1A3 induction therefore links the proposed molecule to a marker with independent therapeutic relevance.

Transcription factors have long been regarded as undruggable because of structural disorder and the absence of defined binding pockets, although direct small-molecule modulation now has precedent.[17] Molecular glues extend that precedent by exploiting cooperative binding at protein interfaces, and a recent review places transcription factors within reach of the approach.[18] The closest mechanistic precedent is the BCL6 ligand BI-3802, which induced polymerization and degradation of a transcription factor through a composite ligand–protein surface.[19] That outcome is degradation, whereas the present framework seeks stabilization of a native assembly, a stricter requirement because the ligand must bind with positive cooperativity without distorting DNA recognition. The cooperativity gate (G3) and the paralog counter-docking stage (G4) were designed to meet it.

Structural modeling is the most vulnerable step of the pipeline, and published benchmarks bear directly on the gate design. AlphaFold-based modeling of transient heterodimeric complexes succeeded in many cases but not uniformly, and success depended on complex type and alignment input,[20] whereas interface confidence scores from AlphaFold2 helped to separate correct from incorrect interaction models.[21] Predicted structures should nevertheless be treated as hypotheses that require experimental confirmation,[22] and docking into predicted models of G protein–coupled receptors produced ligand poses much less accurate than docking into experimental structures.[23] These reports support the scrambled-interface controls and the requirement for public model deposition before screening.

End-point free-energy scoring is similarly approximate. MM/PBSA and MM/GBSA have reproduced experimental trends in some systems, yet they contain crude treatments of conformational entropy and binding-site water,[24] which is why the cooperativity threshold serves to prioritize candidates rather than to predict affinity. PAINS filtering poses a comparable caveat, because it removes recognized interference motifs but cannot certify that a compound is free of assay artifacts, and a substructure alert does not necessarily imply broad interference.[25] Stage 5 triage therefore remains indispensable.

The framework has boundaries that should be stated plainly. The RDKit prototype is a cheminformatic reference and not a validated binder, and no structural model has yet been computed or deposited. Stress-induced post-translational modifications are difficult to represent in standard simulations, and PDX1 expression in duodenal epithelium and exocrine pancreas raises the possibility of off-target effects that only cellular assays can address. Negative gate outcomes are informative: failure of Gate G1 would redirect the search toward the ternary assembly or DNA-bound conformations, and failure of Gate G2 or G3 would end the campaign by an objective rule. A candidate that passes all computational gates would still require biophysical confirmation and cellular testing under glucolipotoxic stress before any in vivo work could be justified.

5. CONCLUSIONS

This work defines a gated computational framework that translates the biological hypothesis of PDX1–MAFA–NKX6.1 complex stabilization into a concrete, decision-driven discovery path. It supplies a three-tier evidence hierarchy, five stages closed by explicit quantitative gates, three experimentally falsifiable predictions, and an open-source cheminformatic reference prototype chemotype parameterized in RDKit. It reports no computed structural models and claims no biological activity. The first operational requirement is the generation, rigorous negative-control evaluation, and public deposition of PDX1–MAFA complex models (Gate G1); only after that foundation is established does the search for molecular glues become scientifically warranted.

Declarations

Competing interests:

The authors declare no competing interests.

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Figures

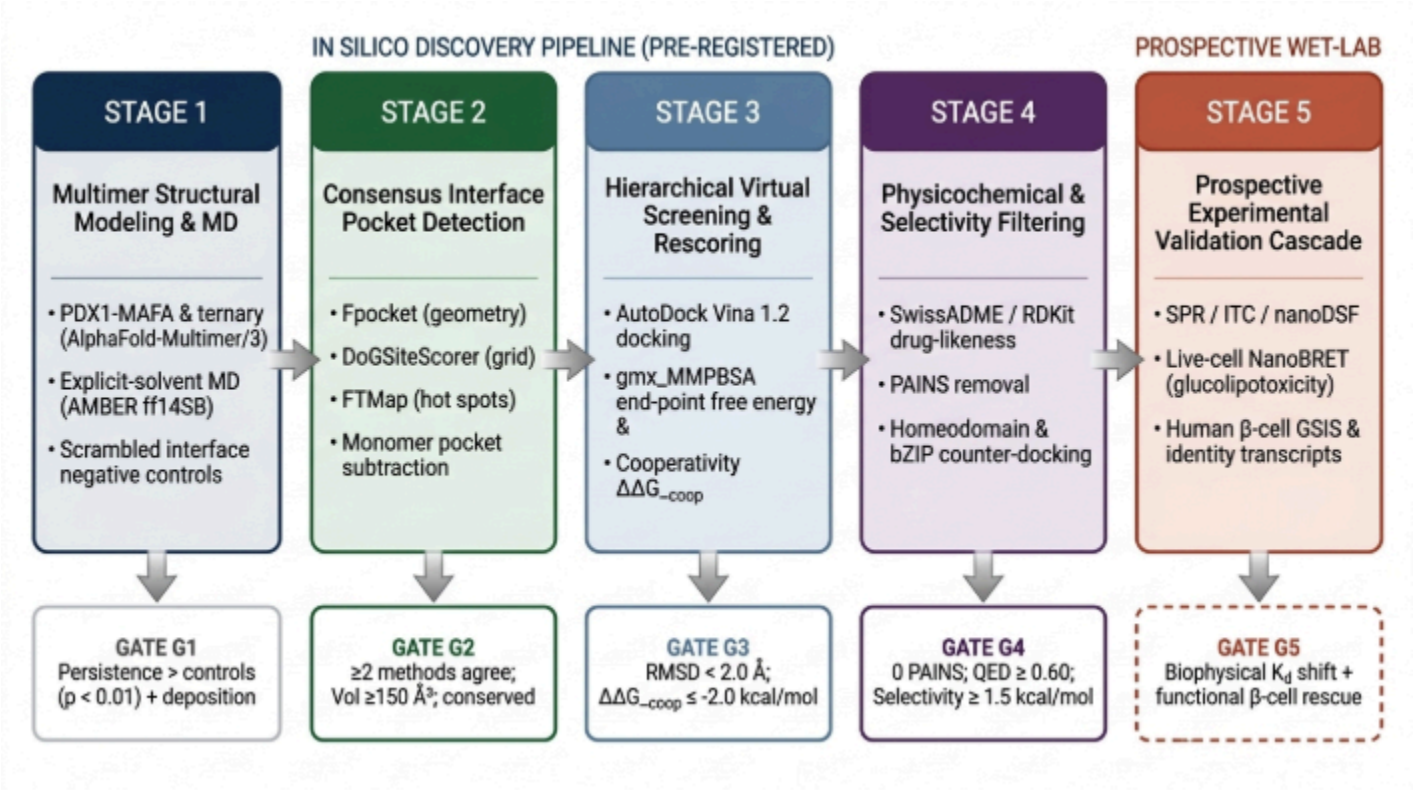


Figure 1

Schematic of the five-stage decision-gated computational and translational discovery framework. Stages 1–4 constitute the preregistered in silico discovery pipeline; Stage 5 represents prospective wet-lab validation. Each stage terminates in an explicit quantitative Go/No-Go decision gate (G1–G5) incorporating orthogonal negative controls.

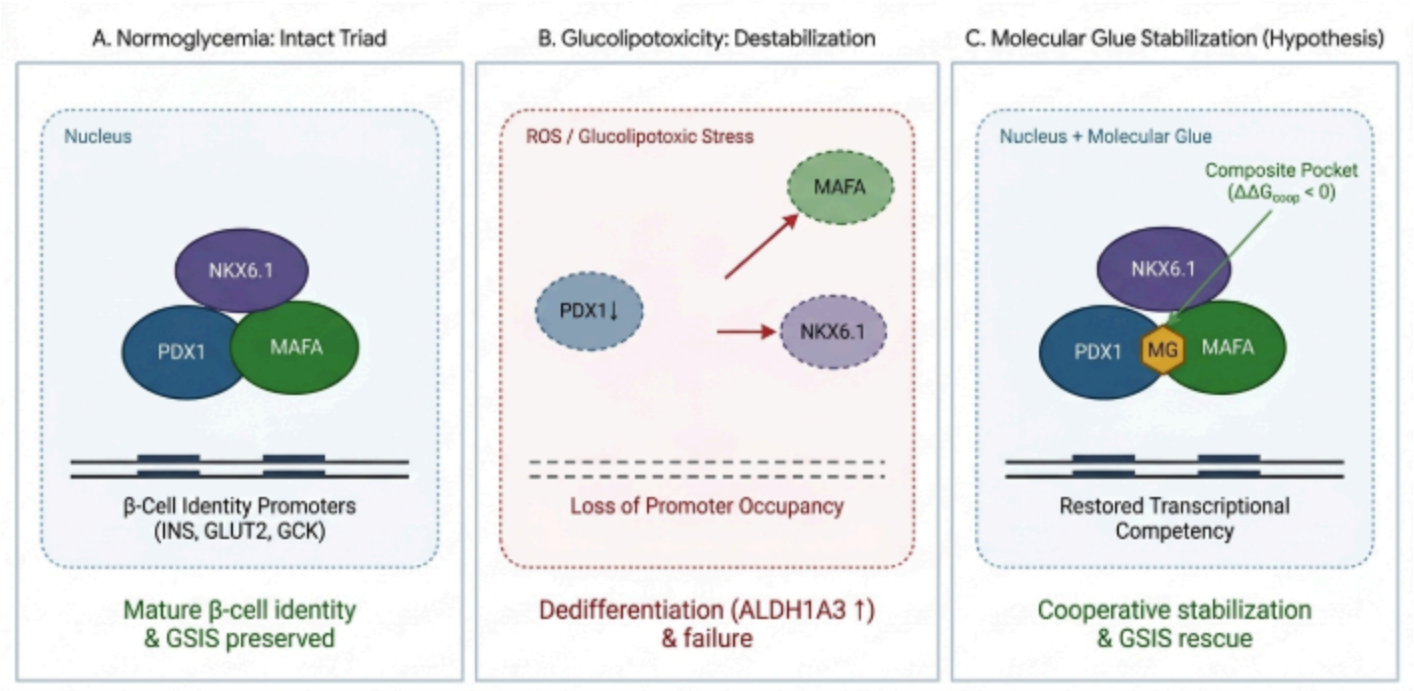


Figure 2

Conceptual model of β-cell transcriptional triad destabilization and molecular glue stabilization. (A) Intact nuclear PDX1–MAFA–NKX6.1 assembly supporting β-cell identity promoters and glucose-stimulated insulin secretion (GSIS). (B) Destabilization and cytoplasmic translocation of MAFA and NKX6.1 with loss of PDX1 occupancy under oxidative and glucolipotoxic stress. (C) Hypothetical cooperative binding of a molecular glue (MG) within a composite pocket at the PDX1–MAFA interface (ΔΔG_coop < 0) to preserve nuclear assembly and secretory function.

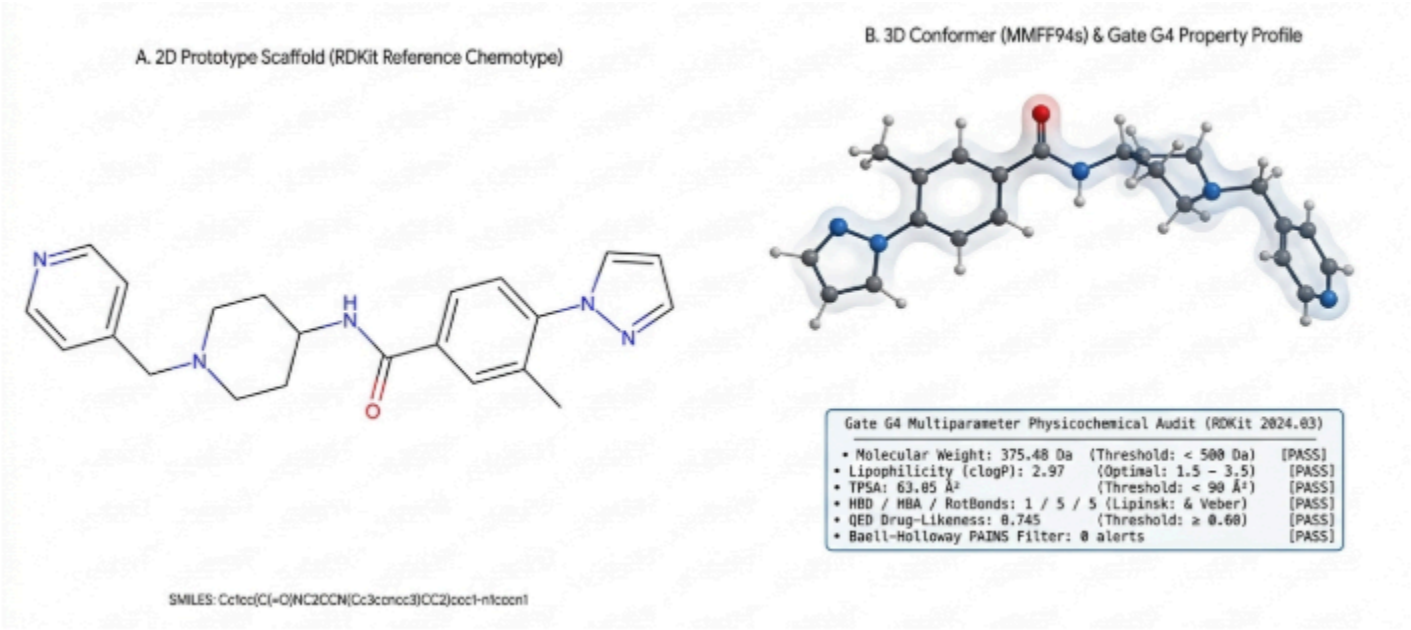


Figure 3

Open-source in silico prototype molecular glue chemotype generated and parameterized in RDKit. (A) Two-dimensional chemical structure of the reference prototype scaffold (N-[1-(pyridin-4-ylmethyl)piperidin-4-yl]-3-methyl-4-(1H-pyrazol-1-yl)benzamide; C22H25N5O). (B) Energy-minimized 3D conformer (ETKDGv3 / MMFF94s force field) and Gate G4 multiparameter physicochemical audit confirming Lipinski/Veber compliance, high drug-likeness (QED = 0.745), and zero PAINS alerts.