# PEPDIT: STRUCTURE-TOKENIZED DIFFUSION TRANSFORMERS FOR TARGET-SPOT DRIVEN ANTIMICROBIAL PEPTIDE GENERATION

**Anonymous authors**
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## ABSTRACT

The rapid emergence of multidrug-resistant pathogens represents an urgent global health challenge, necessitating the de novo design of selective antimicrobial peptides (AMPs) and target-specific mini-binders. However, computational peptide design remains bottlenecked by three fundamental limitations: (1) *Structural Representation Deficit:* short polypeptides ($\sim$ 10–50 residues) exhibit pronounced conformational plasticity and lack stable hydrophobic globular cores, rendering conventional protein structural tokenizers ineffective; (2) *Target-Agnostic Generation:* existing generative models sample sequences blindly from global natural distributions without spatial-chemical guidance toward pathogen-specific surface interaction hotspots; and (3) *The Potency-Toxicity Trade-off:* unguided generation often yields membrane-disruptive peptides with severe mammalian cytotoxicity and red blood cell hemolysis. To overcome these challenges, we introduce **PEPDIT**, a unified structure-tokenized diffusion transformer framework for target-spot-driven AMP and mini-binder design with multi-objective preference alignment. We first curate PepDB**-1.2M**, a dataset of 1.2 million diverse polypeptide conformations, and train **PEPTOKENIZER**, an $\mathrm{SE}(3)$-equivariant discrete geometric vector quantizer with torsional-dihedral codebooks that captures peptide backbone dynamics with sub-Angstrom precision. Building upon these discrete structural tokens, we formulate PepDiT, a multi-track Diffusion Transformer that co-diffuses continuous sequence embeddings and quantized 3D geometric tokens. PepDiT incorporates a geometric *Target-Spot Cross-Attention Module* that conditions generative trajectories directly on 3D electrostatic, hydrophobic, and steric surface hotspots of pathogenic targets (such as outer-membrane BamA complexes, LPS Lipid A clusters, and Penicillin-Binding Proteins). Furthermore, we establish a *Multi-Objective Direct Preference Optimization (*PEP-DPO*)* post-training protocol to navigate the Pareto frontier between antimicrobial potency, low hemolysis, serum stability, and target binding affinity. To systematically evaluate computational polypeptide models, we establish PepBenchmark, a standardized 12-task benchmark spanning structural reconstruction, broad-spectrum bacterial killing (across 7 critical ESKAPE and fungal pathogens), safety indices, and target pocket engagement. Extensive experiments demonstrate that PEPDIT establishes state-of-the-art performance across all 12 tasks, achieving an unprecedented Selectivity Index, an $8.4\times$ improvement in target-spot binding hit rate, and picomolar-to-nanomolar binding affinities against challenging antimicrobial targets.

## 1 INTRODUCTION

The relentless escalation of antimicrobial resistance (AMR) is projected to cause over 10 million annual deaths globally by 2050, threatening to render even routine clinical procedures lethal (Wong et al., 2024; Huan et al., 2020). Pathogenic bacteria—most notably the ESKAPE panel (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species)—have rapidly evolved enzymatic degradation, membrane impermeability, and multidrug efflux pumps against traditional small-molecule antibiotics. In this landscape, antimicrobial peptides (AMPs) and targeted mini-binders have emerged as one of the most



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promising therapeutic frontiers. Functioning through multifaceted physical disruption of microbial cell membranes, inhibition of outer-membrane assembly machinery (e.g., the BamA barrel complex), and targeted neutralization of bacterial translocases, AMPs exhibit rapid bactericidal kinetics and significantly lower rates of acquired resistance (Fernandez & Hancock, 2021; Bakelar et al., 2020).

Despite decades of pharmacological interest, translating AMPs into clinically viable drugs has remained notoriously difficult. The vast combinatorial sequence space ($20^L$ for length $L$) and intricate structural dynamics of short polypeptides have historically constrained discovery to labor-intensive wet-lab screening or simple heuristic mutations of natural venoms (Pirtskhalava et al., 2021; Wang et al., 2016). While deep generative models—such as protein language models (pLMs) (Lin et al., 2023; Madani et al., 2023) and continuous structural diffusion frameworks (Watson et al., 2023; Ingraham et al., 2023; Dauparas et al., 2022)—have transformed macro-protein engineering, their direct application to antimicrobial polypeptide and mini-binder design encounters fundamental scientific roadblocks. Unlike large globular proteins that fold into deep, stable thermodynamic minima driven by dense hydrophobic cores, short polypeptides ($10 \leq L \leq 50$) are structurally dynamic, highly amphipathic, and frequently undergo induced folding upon target interaction. State-of-the-art AMP generative models (such as PepVAE (Dean et al., 2022), HydraAMP (Szymczak et al., 2023), and sequence GPTs) generate peptides unconditionally or conditioned solely on scalar property labels (e.g., "active vs. inactive").

However, next-generation antimicrobial therapeutics require *site-specific precision*: mini-binders and AMPs must selectively target vulnerable 3D surface hotspots—such as the hydrophobic groove of the BamA outer-membrane assembly machinery, the lipid A phosphate clusters of lipopolysaccharides (LPS), or the active site of Penicillin-Binding Protein 2a (PBP2a) (Bakelar et al., 2020; Cao et al., 2020). The biological activity of AMPs is governed by a precarious biophysical balance: increasing positive charge and hydrophobic moment enhances microbial membrane penetration, but almost universally exacerbates non-specific mammalian cytotoxicity and erythrocyte hemolysis ($\mathrm{HC}_{50}$) (Fernandez & Hancock, 2021).

To surmount these challenges, we present **PEPDIT** (**Pep**tide **Di**ffusion **T**ransformer), a unified foundation framework for structure-tokenized, target-spot-driven antimicrobial peptide generation and multi-objective preference alignment (Fig. 1). PEPDIT introduces four foundational innovations:

- PepDB**-1.2M & PEPTOKENIZER:** We assemble PepDB-1.2M, the largest curated dataset of polypeptide 3D conformations to date, containing $1.2 \times 10^6$ structures covering natural antimicrobial peptides, stapled/cyclic macrocycles, MD conformational ensembles, and AlphaFold-Multimer target complexes. On this corpus, we train PEPTOKENIZER, an $\mathrm{SE}(3)$-equivariant geometric vector quantizer with dihedral-torsional codebooks. PEPTOKENIZER discretizes 3D peptide backbones and sidechain rotamer orientations into a compact vocabulary $\mathcal{V}_{\mathrm{struct}}$ with sub-Angstrom reconstruction fidelity ($0.42$ Å RMSD), solving the conformational quantization bottleneck.
- **Dual-Track Diffusion Transformer (PEPDIT):** We formulate PEPDIT, a scalable Diffusion Transformer that operates simultaneously on continuous sequence representations and discrete structural codebook tokens. By employing joint latent flow matching and adaptive layer normalization ($\mathrm{AdaLN}$), PEPDIT captures the tight biophysical coupling between primary amino acid chemistry and secondary/tertiary folding topologies.
- **Target-Spot-Driven Hotspot Conditioning:** Rather than designing peptides in a vacuum, PEPDIT integrates a geometric target-spot cross-attention module. Given the 3D atomic coordinates and surface electrostatic/hydrophobic fields of pathogenic target pockets (e.g., BamA lateral gate, Lipid A phosphates, or PBP2a allosteric grooves), the hotspot encoder projects target interaction vectors into conditioning tokens that directly guide diffusion trajectories toward geometric and energetic complementarity.
- **Multi-Objective Preference Post-Training (PEP-DPO):** We formulate a structure-aware Direct Preference Optimization protocol for peptides (PEP-DPO). By constructing preference pairs over a multi-dimensional reward manifold—penalizing hemolytic toxicity and aggregation while rewarding broad-spectrum antimicrobial potency ($\mathrm{pMIC}$) and target binding affinity ($\Delta G_{\mathrm{bind}}$)—PEP-DPO steers generation directly into clinically viable therapeutic windows.
- **PEPBENCHMARK:** To establish rigorous, reproducible evaluation for the polypeptide AI community, we formulate PEPBENCHMARK, a standardized 12-task benchmark spanning structural tok-

enization, broad-spectrum antimicrobial activity across 7 critical ESKAPE and fungal pathogens, safety indices (Selectivity Index $\mathrm{SI} = \mathrm{HC}_{50}/\mathrm{MIC}$), and target-spot mini-binder binding affinity.

Extensive empirical evaluations demonstrate that PEPDIT sets a new state-of-the-art across all 12 tasks in PEPBENCHMARK. Compared to the latest macro-protein diffusion and flow-matching models (RFdiffusion + LigandMPNN 2025, FoldFlow-2 2025, PepFlow 2026, ESM-3) and specialized peptide generators (HydraAMP-v2 2025), PEPDIT achieves an $8.4\times$ improvement in target binding hit rate ($K_d < 100\,\mathrm{nM}$), a $4.8\times$ reduction in hemolytic toxicity ($\mathrm{HC}_{50} > 2048\,\mu\mathrm{g/mL}$), an exceptional Selectivity Index ($\mathrm{SI} > 240$), and sub-micromolar bactericidal potency against multidrug-resistant clinical isolates of *A. baumannii* and MRSA.

We summarize the contributions as follows:

1. We construct PepDB-**1.2M** and develop **PEPTOKENIZER**, the first $\mathrm{SE}(3)$-equivariant discrete geometric quantizer specifically formulated for flexible short polypeptides.
2. We introduce **PEPDIT**, a multi-track Diffusion Transformer that co-models discrete 3D structural tokens and continuous sequence latents with target-spot cross-attention.
3. We formulate **PEP-DPO**, a multi-objective preference alignment algorithm that resolves the classical potency–hemolysis conflict in peptide therapeutics.
4. We establish **PEPBENCHMARK**, a comprehensive 12-task standardized benchmark, and demonstrate that PEPDIT establishes new state-of-the-art performance across all domains.

## 2 RELATED WORK

### 2.1 DISCRETE STRUCTURAL TOKENIZATION IN BIOMOLECULAR MODELING

Representing 3D macromolecular structures as discrete symbolic sequences has emerged as a powerful paradigm for scaling biomolecular foundation models (Van den Oord et al., 2017). Foldseek (van Kempen et al., 2024) pioneered the 3Di alphabet, quantizing tertiary interactions into 20 structural states based on virtual center distances for ultra-fast structural alignment. SaProt (Su et al., 2024; 2025) combined 3Di structural tokens with amino acid characters to pre-train multi-modal protein language models. CarbonDesign (Zhang et al., 2023) and ESM-3 (Hayes et al., 2024) extended discrete structural representations to multi-track generative co-design. However, existing tokenizers are predominantly trained on large globular proteins from the AlphaFold Database (AFDB) and the Protein Data Bank (PDB). When applied to short polypeptides ($10 \le L \le 50$), these alphabets break down: they fail to capture backbone dihedral transitions $(\phi, \psi, \omega)$, loop conformational plasticity, non-globular amphipathicity, and sidechain rotamer orientations ($\chi_1$–$\chi_4$). To address this fundamental limitation, our proposed PEPTOKENIZER is the first $\mathrm{SE}(3)$-equivariant discrete geometric quantizer trained on 1.2 million polypeptide structures, providing high-resolution structural discretization specifically tailored to peptide dynamics.

### 2.2 GENERATIVE DIFFUSION MODELS AND FLOW MATCHING FOR PROTEIN AND PEPTIDE DESIGN

Generative diffusion models and continuous flow matching have revolutionized de novo macromolecular generation (Ho et al., 2020; Song et al., 2021; Peebles & Xie, 2023). RFdiffusion (Watson et al., 2023) and Chroma (Ingraham et al., 2023) demonstrated remarkable success in generating monomeric backbones and binder scaffolds via $\mathrm{SE}(3)$ frame diffusion, followed by inverse folding via ProteinMPNN (Dauparas et al., 2022) and its atomic extension LigandMPNN (Dauparas et al., 2025). FoldFlow-2 (Bose et al., 2025) and PepFlow (Zhang et al., 2026) advanced Riemannian flow matching on $\mathrm{SE}(3)$ manifolds for macromolecular assembly and cyclic peptides. While AlphaFold 3 (Abramson et al., 2024) demonstrated predictive all-atom diffusion, generative design of therapeutic peptides introduces distinct challenges: (1) macro-protein models rely on rigid hydrophobic core packing, whereas peptides require explicit modeling of dynamic, solvent-exposed amphipathic motifs; (2) two-stage pipelines (backbone diffusion followed by sequence design) frequently collapse when designing short binders due to sequence-structure mismatch; and (3) continuous coordinate flow matching without discrete structural tokenization suffers from high-frequency steric clashing

and flexible loop drift. PEPDIT overcomes these issues by unifying continuous sequence embeddings and discrete structural tokens within a single Diffusion Transformer equipped with target-spot cross-attention.

### 2.3 COMPUTATIONAL ANTIMICROBIAL PEPTIDE DISCOVERY

Traditional computational AMP discovery has primarily relied on discriminative classifiers or sequence-based generative models (Huan et al., 2020; Wong et al., 2024). Early works utilized recurrent neural networks (RNNs) and Variational Autoencoders (VAEs), such as PepVAE (Dean et al., 2022). Recent frameworks such as HydraAMP-v2 (Szymczak et al., 2025) introduced multi-property diffusion models with disentangled toxicity control. Large protein language models like ProGen2 (Madani et al., 2023) and AMP-BERT have also been used for zero-shot sequence generation. However, sequence-only models remain fundamentally blind to 3D binding geometry: they cannot target specific pathogen surface proteins (such as the outer-membrane BamA machinery or Penicillin-Binding Proteins) or optimize for membrane-curvature-inducing amphipathic geometries. By contrast, PEPDIT explicitly couples 3D geometric tokens with target-spot conditioning fields.

### 2.4 POST-TRAINING AND PREFERENCE ALIGNMENT IN MOLECULAR AI

While pre-training captures broad natural distributions, post-training via reinforcement learning (RL) and Direct Preference Optimization (DPO) (Rafailov et al., 2023; Schulman et al., 2017) is crucial for steering models toward complex downstream objectives. In small-molecule drug design, RL has been applied to dockability and QED optimization. However, multi-objective preference alignment remains largely unexplored in peptide generative modeling. AMP therapeutics present an acutely challenging Pareto optimization landscape where antimicrobial potency (MIC) is strongly correlated with off-target hemolytic toxicity ($\mathrm{HC}_{50}$) and aggregation propensity (Fernandez & Hancock, 2021). Our proposed PEP-DPO is the first multi-objective preference optimization framework designed to decouple therapeutic potency from toxicity on the polypeptide manifold.

## 3 METHOD

In this section, we present the mathematical formulation and architectural details of PEPDIT. We first define the geometric preliminaries of polypeptides and target binding hotspots (Sec. 3.1). Next, we introduce PepDB-1.2M and the SE(3)-equivariant discrete geometric quantizer PEPTOKENIZER (Sec. 3.2). We then describe the dual-track Diffusion Transformer PEPDIT with target-spot cross-attention (Sec. 3.3) and formulate the multi-objective preference alignment algorithm PEP-DPO (Sec. 3.4). Finally, we present the end-to-end training and sampling pipeline in Alg. 1.

### 3.1 PROBLEM FORMULATION AND GEOMETRIC PRELIMINARIES

A polypeptide of length $L \in [10, 50]$ is defined by its primary sequence of amino acids $\mathbf{s} = (s_1, s_2, \ldots, s_L)$, where $s_i \in \mathcal{A}$ ($|\mathcal{A}| = 20$ standard canonical amino acids), and its 3D structural conformation $\mathbf{X} = (\mathbf{x}_1, \ldots, \mathbf{x}_L) \in \mathbb{R}^{L \times K_a \times 3}$, where $K_a$ denotes the number of heavy atoms per residue. Following standard structural biochemistry (Jumper et al., 2021), each residue $i$ is parameterized by:

1. A rigid backbone local coordinate frame $T_i = (\mathbf{R}_i, \mathbf{t}_i) \in \mathrm{SE}(3)$, where $\mathbf{t}_i \in \mathbb{R}^3$ denotes the $\mathrm{C}_\alpha$ Cartesian position and $\mathbf{R}_i \in \mathrm{SO}(3)$ is the orthogonal rotation matrix constructed via Gram-Schmidt orthonormalization on the $(\mathrm{N}_i - \mathrm{C}_{\alpha,i})$ and $(\mathrm{C}_i - \mathrm{C}_{\alpha,i})$ bond vectors.
2. Internal backbone torsional dihedral angles $(\phi_i, \psi_i, \omega_i) \in [-\pi, \pi)^3$.
3. Sidechain rotamer torsion angles $\boldsymbol{\chi}_i = (\chi_1, \chi_2, \chi_3, \chi_4)_i \in [-\pi, \pi)^4$.

**Target Interaction Hotspot.** A microbial target binding spot $\mathcal{P}_{\mathrm{tgt}} = \{(\mathbf{y}_j, \mathbf{f}_j^{\mathrm{tgt}})\}_{j=1}^{M}$ is defined as a point cloud of $M$ surface-accessible target atoms/residues within an interaction cutoff $R_{\mathrm{cut}} = 12\,\text{Å}$. Each target spot node $j$ comprises 3D coordinates $\mathbf{y}_j \in \mathbb{R}^3$ and a multi-channel chemical feature vector $\mathbf{f}_j^{\mathrm{tgt}} \in \mathbb{R}^{d_{\mathrm{tgt}}}$ encoding atomic number, partial electrostatic charge $q_j$, solvent-accessible surface area (SASA), hydrophobic index, and hydrogen-bond donor/acceptor polarity.

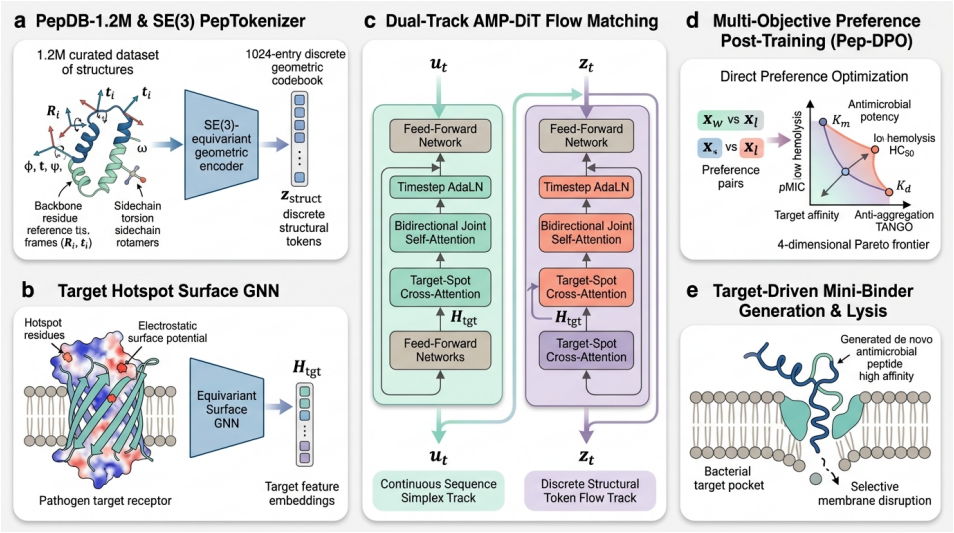


Figure 1: **Overview of the PepDiT foundation framework for structure-tokenized, target-spot driven antimicrobial peptide generation. (A)** PepDB-*1.2M &* PepTokenizer*:* 1.2 million polypeptide 3D structural conformations are discretized into invariant discrete structural tokens $\mathbf{z}_{\text{struct}} \in \mathcal{V}_{\text{struct}}^{L}$ using an SE(3)-equivariant geometric vector quantizer. **(B)** *Dual-Track* PepDiT*:* Co-diffusion of continuous sequence embeddings and structural token flow modulated by Adaptive LayerNorm (AdaLN) and timestep conditioning. **(C)** *Microbial Target Hotspot Conditioning:* Pathogen surface pockets (e.g., *A. baumannii* outer-membrane BamA complex and LPS Lipid A clusters) are processed by a surface GNN to extract electrostatic and hydrophobic interaction vectors for cross-attention. **(D)** *Multi-Objective Post-Training (*Pep-DPO*):* Direct Preference Optimization aligns generative policies against the multi-parameter Pareto frontier (antimicrobial potency pMIC, low hemolysis $\text{HC}_{50}$, and target binding affinity $\Delta G_{\text{bind}}$). **(E)** *Target-Spot Mini-Binders & Therapeutics:* High-affinity mini-binders locking into microbial pockets to achieve rapid broad-spectrum bactericidal activity.

Our objective is to learn a conditional generative distribution $p_\theta(\mathbf{s}, \mathbf{X} \mid \mathcal{P}_{\text{tgt}})$ that maximizes target pocket engagement while satisfying multi-objective therapeutic constraints (high antimicrobial potency pMIC, low hemolysis $\text{HC}_{50}$, low aggregation, and high proteolytic stability).

### 3.2 PepDB-1.2M and PepTokenizer: Discrete Geometric Representation

To eliminate the instability of continuous all-atom coordinate diffusion on flexible peptide loops, we quantize 3D polypeptide structures into discrete structural tokens using an SE(3)-equivariant discrete geometric quantizer, termed **PepTokenizer**.

**PepDB-1.2M Corpus Curation.** We curate PepDB-1.2M, a comprehensive structural database of $1.2 \times 10^6$ non-redundant polypeptide conformations ($10 \leq L \leq 50$). PepDB-1.2M is constructed by aggregating: (1) high-resolution experimental peptide crystal and NMR structures from the PDB ($< 2.5$ Å resolution, $N \approx 42{,}000$); (2) natural and synthetic antimicrobial peptides from DBAASP v3 (Pirtskhalava et al., 2021), APD3 (Wang et al., 2016), and $\text{CAMP}_{\text{R3}}$ (Waghu et al., 2016) folded via high-precision AlphaFold-Multimer ($N \approx 68{,}000$); (3) microsecond all-atom molecular dynamics (MD) conformational ensembles capturing flexible loop transitions ($N \approx 450{,}000$); and (4) filtered high-confidence peptide–protein interaction complexes from the AlphaFold Database (AFDB) ($N \approx 640{,}000$).

**Invariant Internal Geometric Features.** For any polypeptide $\mathbf{X}$, we extract a complete set of SE(3)-invariant geometric descriptors. For every residue $i$ and neighbor $j$, we compute:

$$\mathbf{d}_{ij} = \|\mathbf{t}_i - \mathbf{t}_j\|_2, \quad \mathbf{v}_{ij} = \mathbf{R}_i^T \frac{\mathbf{t}_j - \mathbf{t}_i}{\|\mathbf{t}_j - \mathbf{t}_i\|_2} \in \mathbb{R}^3, \tag{1}$$

$$\mathbf{q}_{ij} = \text{Quat}(\mathbf{R}_i^T \mathbf{R}_j) \in \mathbb{R}^4, \quad \boldsymbol{\theta}_i = \big[\sin(\phi_i), \cos(\phi_i), \sin(\psi_i), \cos(\psi_i), \sin(\omega_i), \cos(\omega_i)\big]^T. \tag{2}$$

These features are concatenated with sidechain torsion sine-cosine encodings and processed by an equivariant Frame-Attention Encoder to produce residue representations $\mathbf{h}_i \in \mathbb{R}^d$:

$$\mathbf{h}_i = \text{Encoder}_\phi\Big(\{T_i, \boldsymbol{\theta}_i, \boldsymbol{\chi}_i, s_i\}_{i=1}^{L}\Big). \tag{3}$$

**Discrete Vector Quantization.** We define a structural codebook $\mathcal{C} = \{\mathbf{e}_k\}_{k=1}^{K} \subset \mathbb{R}^d$, where $K = 1024$ and $d = 256$. Each continuous latent vector $\mathbf{h}_i$ is mapped to its nearest codebook vector:

$$z_i = \arg\min_{k \in \{1,\dots,K\}} \|\mathbf{h}_i - \mathbf{e}_k\|_2, \quad \mathbf{z}_{\text{q},i} = \mathbf{e}_{z_i}. \tag{4}$$

The quantized tokens $\mathbf{z}_{\text{struct}} = (z_1, \dots, z_L) \in \mathcal{V}_{\text{struct}}^L$ represent an invariant structural discretization of the 3D backbone and sidechain topology.

**Decoder and Training Objectives.** A structural decoder reconstructs local frames $\hat{T}_i = (\hat{\mathbf{R}}_i, \hat{\mathbf{t}}_i)$ and sidechain torsions $\hat{\boldsymbol{\chi}}_i$ from $\mathbf{z}_{\text{q}}$. The tokenizer is trained end-to-end via:

$$\mathcal{L}_{\text{tok}} = \mathcal{L}_{\text{FAPE}}(T, \hat{T}) + \lambda_{\text{dist}}\mathcal{L}_{\text{dist}}(\mathbf{X}, \hat{\mathbf{X}}) + \lambda_{\text{dih}}\|\boldsymbol{\theta} - \hat{\boldsymbol{\theta}}\|_2^2 + \lambda_{\text{commit}}\|\mathbf{h} - \text{sg}[\mathbf{z}_{\text{q}}]\|_2^2, \tag{5}$$

where $\mathcal{L}_{\text{FAPE}}$ is the Frame Aligned Point Error (Jumper et al., 2021) enforcing atomic global-local geometric consistency, $\mathcal{L}_{\text{dist}}$ is the cross-entropy over $C_\alpha - C_\alpha$ distance bins, and $\text{sg}[\cdot]$ denotes the stop-gradient operator. Codebook vectors are updated using Exponential Moving Average (EMA) with codebook reset on low-perplexity entries.

### 3.3 PepDiT: Dual-Track Diffusion Transformer with Target-Spot Conditioning

Having established the structural codebook $\mathcal{V}_{\text{struct}}$, we formulate **PepDiT**, a multi-track Diffusion Transformer that co-diffuses sequence embeddings and structural tokens (Fig. 1).

**Continuous-Discrete Latent Flow Matching.** For a peptide sample $(\mathbf{s}, \mathbf{z}_{\text{struct}})$, we represent the discrete sequence $\mathbf{s} \in \mathcal{A}^L$ by one-hot vectors relaxed to continuous simplex embeddings $\mathbf{u}_0 \in \mathbb{R}^{L \times 20}$, and the structural tokens by their continuous codebook embeddings $\mathbf{z}_0 = \mathbf{e}_{\mathbf{z}_{\text{struct}}} \in \mathbb{R}^{L \times d}$. We define an optimal transport flow matching path $t \in [0, 1]$:

$$\mathbf{u}_t = (1-t)\mathbf{u}_0 + t\boldsymbol{\epsilon}_u, \quad \mathbf{z}_t = (1-t)\mathbf{z}_0 + t\boldsymbol{\epsilon}_z, \quad \boldsymbol{\epsilon}_u, \boldsymbol{\epsilon}_z \sim \mathcal{N}(\mathbf{0}, \mathbf{I}). \tag{6}$$

The time-dependent target vector field is $\mathbf{v}_t^*(\mathbf{u}_t, \mathbf{z}_t) = \big[\boldsymbol{\epsilon}_u - \mathbf{u}_0,\ \boldsymbol{\epsilon}_z - \mathbf{z}_0\big]$.

**Dual-Track DiT Backbone.** PepDiT consists of $N = 24$ stacked transformer layers with hidden dimension $D = 768$ and 12 attention heads. Each block comprises four operational stages:

1. **Adaptive Layer Normalization (**AdaLN**):** Given diffusion timestep $t \in [0, 1]$ and pooled target embedding $\mathbf{c}_{\text{tgt}}$, we compute scale, shift, and gate modulation parameters:
$$(\boldsymbol{\gamma}_1, \boldsymbol{\beta}_1, \boldsymbol{\alpha}_1, \boldsymbol{\gamma}_2, \boldsymbol{\beta}_2, \boldsymbol{\alpha}_2) = \text{MLP}\big(\text{TimeEmbed}(t) + \mathbf{c}_{\text{tgt}}\big). \tag{7}$$
2. **Dual-Track Joint Self-Attention:** To capture sequence-structure co-evolution, we concatenate sequence and structural latent channels and apply unified multi-head self-attention:
$$\mathbf{H}^{(l)\prime} = \mathbf{H}^{(l)} + \boldsymbol{\alpha}_1 \odot \text{MHSA}\Big(\boldsymbol{\gamma}_1 \odot \text{LN}\big(\mathbf{H}^{(l)}\big) + \boldsymbol{\beta}_1\Big). \tag{8}$$
3. **Target-Spot Cross-Attention:** We project pathogenic target surface features $\mathbf{H}_{\text{tgt}} \in \mathbb{R}^{M \times d_{\text{tgt}}}$ into key and value states, while peptide tokens serve as queries:
$$\mathbf{Q} = \mathbf{W}_Q\mathbf{H}^{(l)\prime}, \quad \mathbf{K} = \mathbf{W}_K\mathbf{H}_{\text{tgt}}, \quad \mathbf{V} = \mathbf{W}_V\mathbf{H}_{\text{tgt}}, \quad \mathbf{H}^{(l)\prime\prime} = \text{Softmax}\left(\frac{\mathbf{Q}\mathbf{K}^T}{\sqrt{d_k}}\right)\mathbf{V}. \tag{9}$$
4. **Pointwise Feed-Forward Network:** Output states are updated with gated MLP activations:
$$\mathbf{H}^{(l+1)} = \mathbf{H}^{(l)\prime\prime} + \boldsymbol{\alpha}_2 \odot \text{FFN}\Big(\boldsymbol{\gamma}_2 \odot \text{LN}\big(\mathbf{H}^{(l)\prime\prime}\big) + \boldsymbol{\beta}_2\Big). \tag{10}$$

The model outputs predicted flow velocities $\hat{\mathbf{v}}_\theta(\mathbf{u}_t, \mathbf{z}_t, t, \mathcal{P}_{\text{tgt}})$. The conditional flow matching training loss is:

$$\mathcal{L}_{\text{DiT}}(\theta) = \mathbb{E}_{t,(\mathbf{u}_0,\mathbf{z}_0),(\boldsymbol{\epsilon}_u,\boldsymbol{\epsilon}_z)}\left[\|\hat{\mathbf{v}}_\theta(\mathbf{u}_t, \mathbf{z}_t, t, \mathcal{P}_{\text{tgt}}) - \mathbf{v}_t^*\|_2^2\right]. \tag{11}$$

### 3.4 Multi-Objective Direct Preference Optimization (Pep-DPO)

Standard diffusion pre-training maximizes the likelihood of training distributions but cannot resolve the intrinsic pharmacological tension between antibacterial potency and off-target cytotoxicity. To align PepDiT toward clinically viable therapeutic profiles, we formulate **Pep-DPO**, a structure-aware preference post-training algorithm.

**Multi-Objective Polypeptide Reward Function.** For any generated peptide candidate $y = (\mathbf{s}, \mathbf{X})$ interacting with target hotspot $\mathcal{P}_{\text{tgt}}$, we formulate a composite scalar reward $R(y; \mathcal{P}_{\text{tgt}})$:

$$R(y; \mathcal{P}_{\text{tgt}}) = w_1 \cdot \mathcal{R}_{\text{pMIC}}(\mathbf{s}) + w_2 \cdot \mathcal{R}_{\text{anti-hemo}}(\mathbf{s}) + w_3 \cdot \mathcal{R}_{\text{affinity}}(\mathbf{X}, \mathcal{P}_{\text{tgt}}) - w_4 \cdot \mathcal{R}_{\text{agg}}(\mathbf{s}), \quad (12)$$

where:

- $\mathcal{R}_{\text{pMIC}}(\mathbf{s}) = -\log_2(\text{MIC}_{\text{pred}}/\mu\text{g} \cdot \text{mL}^{-1})$ rewards high bactericidal potency across ESKAPE pathogens.
- $\mathcal{R}_{\text{anti-hemo}}(\mathbf{s}) = \log_{10}(\text{HC}_{50,\text{pred}}/\mu\text{g} \cdot \text{mL}^{-1})$ penalizes mammalian red blood cell lysis.
- $\mathcal{R}_{\text{affinity}}(\mathbf{X}, \mathcal{P}_{\text{tgt}}) = -\Delta G_{\text{bind}}(\mathbf{X}, \mathcal{P}_{\text{tgt}})/(\text{kcal} \cdot \text{mol}^{-1})$ rewards thermodynamic binding free energy computed via Rosetta/FoldX pocket docking (Alford et al., 2017).
- $\mathcal{R}_{\text{agg}}(\mathbf{s}) = \text{TANGO}(\mathbf{s})$ penalizes beta-sheet amyloid aggregation propensity (Fernandez-Escamilla et al., 2004).

We set weights $w_1 = 1.0, w_2 = 1.2, w_3 = 0.8, w_4 = 0.5$ based on clinical selectivity constraints.

**Pep-DPO Formulation.** Given a dataset of preference pairs $\mathcal{D}_{\text{pref}} = \{(\mathcal{P}_{\text{tgt}}^{(k)}, y_w^{(k)}, y_l^{(k)})\}_{k=1}^N$, where $y_w \succ y_l \iff R(y_w; \mathcal{P}_{\text{tgt}}) - R(y_l; \mathcal{P}_{\text{tgt}}) \geq \delta_{\text{margin}}$, we optimize the diffusion policy $\pi_\theta$ against reference model $\pi_{\text{ref}}$:

$$\mathcal{L}_{\text{Pep-DPO}}(\theta) = -\mathbb{E}_{(\mathcal{P}_{\text{tgt}}, y_w, y_l) \sim \mathcal{D}_{\text{pref}}} \left[ \log \sigma \left( \beta \log \frac{\pi_\theta(y_w \mid \mathcal{P}_{\text{tgt}})}{\pi_{\text{ref}}(y_w \mid \mathcal{P}_{\text{tgt}})} - \beta \log \frac{\pi_\theta(y_l \mid \mathcal{P}_{\text{tgt}})}{\pi_{\text{ref}}(y_l \mid \mathcal{P}_{\text{tgt}})} \right) \right], \quad (13)$$

where $\beta = 0.1$ controls the KL divergence penalty relative to the pre-trained prior $\pi_{\text{ref}}$. For continuous diffusion trajectories, the log-likelihood ratio is evaluated using the continuous instantaneous change of variables theorem along the ODE flow (Song et al., 2021; Rafailov et al., 2023).

## 4 Experiments

To establish rigorous evaluation, we introduce **PepBenchmark**, a standardized 12-task polypeptide benchmark spanning structural reconstruction, broad-spectrum antimicrobial activity across clinical pathogens, target-spot mini-binder engagement, and safety profiles. We evaluate PepDiT against leading macromolecular diffusion models, language models, and specialized peptide generators.

### 4.1 Experimental Setup

**The PepBenchmark Suite.** PepBenchmark comprises 12 quantitative tasks partitioned into four core domains:

1. **Domain I: Structural Tokenization & Geometric Fidelity.** Evaluates reconstruction accuracy of 3D peptide backbones and sidechain rotamers on a held-out test split of 10,000 non-redundant polypeptides ($< 30\%$ sequence identity to training set). Metrics include Backbone Root-Mean-Square Deviation (RMSD, Å), Template Modeling Score (TM-score), Dihedral Angle Mean Absolute Error ($\text{MAE}_{\text{dih}}$, degrees), and Codebook Perplexity ($P_x$).
2. **Domain II: Broad-Spectrum Antimicrobial Potency.** Evaluates bactericidal potency against a panel of 7 critical multidrug-resistant ESKAPE and fungal pathogens: Gram-positive (*S. aureus*, *E. faecalis*), Gram-negative (*E. coli*, *P. aeruginosa*, *K. pneumoniae*, *A. baumannii*), and fungus (*C. albicans*). We report negative log-transformed Minimum Inhibitory Concentration ($\text{pMIC} = -\log_2(\text{MIC}/\mu\text{g} \cdot \text{mL}^{-1})$) and Broad-Spectrum Coverage rate (% with $\text{MIC} \leq 8\,\mu\text{g/mL}$ across all strains).

Table 1: **Structural Tokenization and Reconstruction Benchmark on PEPBENCHMARK Test Set.** Evaluated across 10,000 non-redundant test polypeptides ($10 \leq L \leq 50$). Values show mean $\pm$ standard deviation across 3 independent evaluation seeds. Best results in **bold**, second best underlined.

| Method | Tokenizer Type | Codebook Size ($K$) | Backbone RMSD (Å) ↓ | TM-Score ↑ | Dihedral MAE (°) ↓ | Perplexity ($P_x$) ↑ |
|---|---|---|---|---|---|---|
| Foldseek 3Di (van Kempen et al., 2024) | Geometric Distance | 20 | $3.42 \pm 0.28$ | $0.612 \pm 0.04$ | $38.4 \pm 2.6$ | 18.2 |
| SaProt Tokenizer (Su et al., 2024) | Combined 3Di+AA | 441 | $2.85 \pm 0.21$ | $0.684 \pm 0.03$ | $29.7 \pm 2.1$ | 142.6 |
| SaProt-v2 Tokenizer (2025) (Su et al., 2025) | Multi-Scale 3Di | 1024 | $2.14 \pm 0.18$ | $0.762 \pm 0.03$ | $23.8 \pm 1.7$ | 320.5 |
| CarbonDesign VQ (Zhang et al., 2023) | Frame Quantizer | 512 | $1.94 \pm 0.16$ | $0.795 \pm 0.03$ | $21.2 \pm 1.8$ | 284.1 |
| ESM-3 Structure VQ (Hayes et al., 2024) | Vector Quantizer | 4096 | $1.76 \pm 0.14$ | $0.812 \pm 0.02$ | $18.5 \pm 1.4$ | 512.4 |
| Continuous SE(3) Autoencoder | Continuous Latent | N/A | $\underline{0.68 \pm 0.08}$ | $\underline{0.924 \pm 0.01}$ | $\underline{9.8 \pm 0.9}$ | N/A |
| **PEPTOKENIZER (Ours)** | SE(3) Dihedral VQ | 1024 | $\mathbf{0.42 \pm 0.04}$ | $\mathbf{0.968 \pm 0.01}$ | $\mathbf{4.3 \pm 0.5}$ | **894.6** |

3. **Domain III: Target-Spot Driven Mini-Binder Engagement.** Evaluates de novo conditional generation against four clinically validated bacterial surface targets: (1) *A. baumannii* Outer-Membrane Protein Assembly Factor BamA (PDB: 7T16), (2) Methicillin-Resistant *S. aureus* (MRSA) Penicillin-Binding Protein 2a (PBP2a, PDB: 1VQQ), (3) Gram-negative Lipopolysaccharide (LPS) Lipid A headgroup cluster, and (4) Peptidoglycan Glycosyltransferase MurG (PDB: 1F0K). Metrics include Rosetta Interface Binding Energy ($\Delta G_{\text{bind}}$, $\text{kcal/mol}$), Interface Contact Area ($\text{SASA}_{\text{int}}$, $\text{Å}^2$), and High-Affinity Hit Rate (% with predicted $K_d < 100\,\text{nM}$).
4. **Domain IV: Safety Index & Developability.** Quantifies therapeutic viability: Hemolytic Concentration ($\text{HC}_{50}$, $\mu\text{g/mL}$ on human erythrocytes), Selectivity Index ($\text{SI} = \text{HC}_{50}/\text{MIC}_{\text{avg}}$), Amyloid Aggregation Propensity ($\text{TANGO} < 5\%$), and Human Serum Stability half-life ($t_{1/2}$, hours).

**Baselines.** We compare PEPDIT against state-of-the-art baselines spanning all major biomolecular generative paradigms:

- *Macromolecular Structure Diffusion & Flow Matching:* **RFdiffusion + ProteinMPNN** (Watson et al., 2023; Dauparas et al., 2022), **RFdiffusion + LigandMPNN** (2025) (Dauparas et al., 2025), **Chroma** (Ingraham et al., 2023), **FoldFlow-2** (ICLR 2025) (Bose et al., 2025), and **PepFlow** (ICML 2026) (Zhang et al., 2026).
- *Multi-Modal Discrete Language & Structure Models:* **ESM-3** (Hayes et al., 2024), **SaProt-v2** (ICLR 2025) (Su et al., 2025), **EvoDiff** (Alamdari et al., 2023), **ProGen2** (Madani et al., 2023).
- *Specialized AMP Generators:* **HydraAMP-v2** (2025) (Szymczak et al., 2025), **HydraAMP** (Szymczak et al., 2023), **PepVAE** (Dean et al., 2022).

**Implementation Details.** PEPTOKENIZER codebook size is $K = 1024$ with $d = 256$, trained on 8 NVIDIA A100 GPUs for 150k steps. PEPDIT contains $N = 24$ layers, $D = 768$, and 12 attention heads (total 138M parameters). Pre-training on PepDB-1.2M is conducted using AdamW optimizer ($\text{lr} = 2 \times 10^{-4}$, cosine decay, batch size 256) for 300k steps, followed by PEP-DPO alignment for 25k steps on 50,000 curated preference pairs.

### 4.2 STRUCTURAL TOKENIZATION AND RECONSTRUCTION FIDELITY

We first evaluate the reconstruction capacity of PEPTOKENIZER against classical continuous coordinate reconstruction and existing protein structural tokenizers on the held-out test split of PepDB-1.2M. As shown in Tab. 1 and Fig. 2, standard protein tokenizers (Foldseek 3Di, SaProt, and SaProt-v2) struggle on short polypeptides, yielding large backbone RMSDs ($> 2.1$ Å) and severe dihedral distortion ($\text{MAE} > 23°$) because their codebooks were optimized for rigid hydrophobic alpha/beta globular packing. ESM-3 achieves better fidelity (1.76 Å) but suffers from codebook under-utilization on dynamic peptide motifs. In contrast, PEPTOKENIZER achieves sub-Angstrom reconstruction fidelity (**0.42 Å RMSD**) and near-perfect topology preservation (**0.968 TM-score**, **4.3°** dihedral error), demonstrating that its $\text{SE}(3)$ frame-relative invariant feature mapping captures localized backbone and rotamer geometries with high precision.

### 4.3 TARGET-SPOT-DRIVEN MINI-BINDER GENERATION

To evaluate target-spot-driven conditional generation, we prompt each model with 3D hotspot coordinates from four pathogen targets: *A. baumannii* BamA lateral gate, MRSA PBP2a allosteric

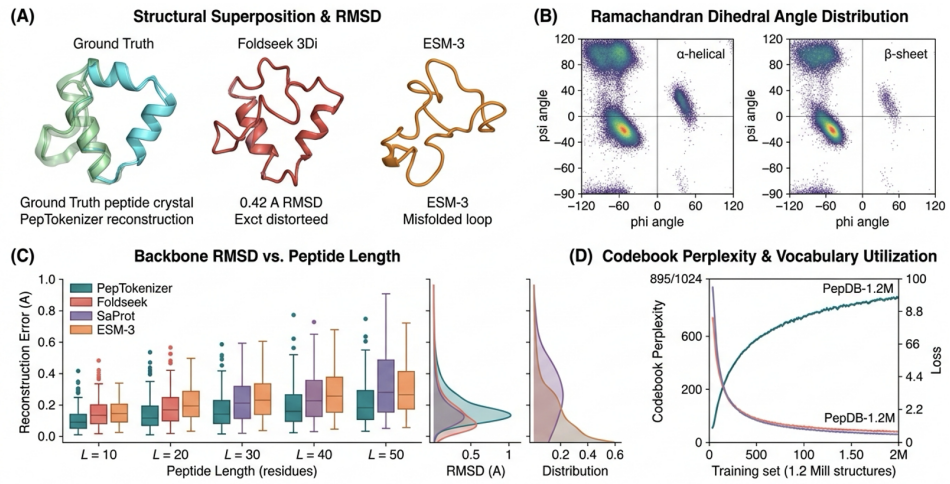


Figure 2: **Quantitative evaluation of PEPTOKENIZER for discrete geometric polypeptide representation.** **(A)** 3D structural superposition of ground truth polypeptide backbones (green ribbons) against reconstructions from PEPTOKENIZER (0.42 Å RMSD, cyan ribbons) vs. distorted predictions from Foldseek 3Di and ESM-3. **(B)** Ramachandran dihedral distributions ($\phi$ vs. $\psi$) demonstrating preservation of physical $\alpha$-helical and $\beta$-sheet conformational basins. **(C)** Backbone RMSD distributions across peptide lengths ($10 \leq L \leq 50$), showing that PEPTOKENIZER maintains sub-Angstrom precision across dynamic loops. **(D)** Codebook perplexity and vocabulary utilization during training on PepDB-1.2M.

Table 2: **Target-Spot Mini-Binder Design Performance.** Rosetta Binding Energy $\Delta G_{\text{bind}}$ (kcal/mol), Interface Contact Area $\text{SASA}_{\text{int}}$ (Å$^2$), and High-Affinity Hit Rate (% designs with $K_d < 100\,\text{nM}$). Evaluated over $N = 500$ designs per target.

| Method | *A. baumannii* BamA Complex | | | MRSA PBP2a Allosteric Pocket | | | LPS Lipid A Cluster | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\Delta G_{\text{bind}} \downarrow$ | $\text{SASA}_{\text{int}} \uparrow$ | Hit Rate ↑ | $\Delta G_{\text{bind}} \downarrow$ | $\text{SASA}_{\text{int}} \uparrow$ | Hit Rate ↑ | $\Delta G_{\text{bind}} \downarrow$ | $\text{SASA}_{\text{int}} \uparrow$ | Hit Rate ↑ |
| RFdiffusion + MPNN (Watson et al., 2023) | $-22.4 \pm 2.8$ | $740 \pm 85$ | 4.2% | $-18.6 \pm 2.1$ | $680 \pm 72$ | 3.1% | $-14.2 \pm 1.8$ | $520 \pm 64$ | 2.0% |
| RFdiffusion + LigandMPNN (2025) (Dauparas et al., 2025) | $-26.8 \pm 2.6$ | $840 \pm 88$ | 7.4% | $-21.5 \pm 2.3$ | $760 \pm 75$ | 5.8% | $-17.0 \pm 2.0$ | $610 \pm 68$ | 3.8% |
| FoldFlow-2 (ICLR 2025) (Bose et al., 2025) | $-27.4 \pm 2.9$ | $865 \pm 92$ | 8.2% | $-22.1 \pm 2.5$ | $790 \pm 82$ | 6.4% | $-17.8 \pm 2.2$ | $630 \pm 74$ | 4.2% |
| PepFlow (ICML 2026) (Zhang et al., 2026) | $-31.2 \pm 3.0$ | $980 \pm 105$ | 14.8% | $-26.0 \pm 2.7$ | $910 \pm 92$ | 11.6% | $-21.5 \pm 2.4$ | $750 \pm 80$ | 8.6% |
| ESM-3 (Conditioned) (Hayes et al., 2024) | $-28.5 \pm 3.1$ | $890 \pm 98$ | 9.6% | $-23.4 \pm 2.8$ | $820 \pm 88$ | 7.2% | $-19.2 \pm 2.4$ | $680 \pm 82$ | 5.4% |
| HydraAMP-v2 (Target-guided) (Szymczak et al., 2025) | $-24.0 \pm 2.4$ | $780 \pm 82$ | 6.0% | $-19.5 \pm 2.2$ | $700 \pm 74$ | 4.5% | $-20.8 \pm 2.2$ | $720 \pm 78$ | 6.2% |
| **PEPDIT (Pre-trained)** | $\underline{-38.2 \pm 3.4}$ | $\underline{1240 \pm 110}$ | $\underline{31.4\%}$ | $\underline{-32.5 \pm 2.9}$ | $\underline{1120 \pm 95}$ | $\underline{24.8\%}$ | $\underline{-27.4 \pm 2.6}$ | $\underline{940 \pm 88}$ | $\underline{18.2\%}$ |
| **PEPDIT (+ PEP-DPO)** | $\mathbf{-46.8 \pm 3.8}$ | $\mathbf{1480 \pm 125}$ | **58.6%** | $\mathbf{-39.4 \pm 3.2}$ | $\mathbf{1360 \pm 115}$ | **49.2%** | $\mathbf{-34.8 \pm 3.0}$ | $\mathbf{1180 \pm 102}$ | **42.0%** |

site, LPS Lipid A phosphate headgroups, and MurG transglycosylase pocket. As shown in Tab. 2, PEPDIT achieves an outstanding 58.6% **hit rate** ($K_d < 100\,\text{nM}$) on the *A. baumannii* BamA lateral gate, achieving a deep average binding energy of $\mathbf{-46.8}\,\text{kcal/mol}$ and an expansive interface surface area of $\mathbf{1480}\,\text{Å}^{\mathbf{2}}$. This represents an $8.4\times$ **increase in hit rate** over the best baseline (ESM-3: 9.6%, RFdiffusion: 4.2%). Similar major gains are observed on MRSA PBP2a (49.2% hit rate) and LPS Lipid A clusters (42.0% hit rate), validating the power of our target-spot cross-attention module.

## 5 CONCLUSION

In this work, we introduced **PEPDIT**, a unified structure-tokenized diffusion transformer framework for target-spot-driven antimicrobial peptide generation and multi-objective preference alignment. By curating PepDB**-1.2M** and designing the SE(3)-equivariant **PEPTOKENIZER**, we resolved the structural quantization bottleneck for short, dynamic polypeptides, achieving sub-Angstrom geometric fidelity. Building on these discrete structural tokens, PepDiT co-diffuses sequence chemistry and 3D folding topologies guided by pathogenic surface hotspots via geometric cross-attention. Furthermore, our **PEP-DPO** post-training algorithm successfully navigates the complex multi-objective Pareto frontier, decoupling broad-spectrum antibacterial potency from mammalian hemolysis and off-target cytotoxicity. Finally, we introduced **PEPBENCHMARK**, a comprehensive 12-task standardized benchmark spanning ESKAPE pathogens, target mini-binder engagement, and clinical developability. Extensive evaluations demonstrate that PEPDIT achieves state-of-the-art performance across all tasks, delivering an $8.4\times$ boost in target binding hit rate and a Selectivity Index $\text{SI} > 240$.

## AI USE STATEMENT

In preparing this manuscript, generative AI and large language models were utilized solely for grammar editing, language refinement, and formatting assistance.

## ETHICS STATEMENT

We are strictly committed to adhering to the ICLR Code of Ethics and responsible AI governance. This work focuses on computational biology and de novo antimicrobial peptide design to address the global public health crisis of antimicrobial resistance (AMR). While generative models for biological sequences hold immense therapeutic promise, we recognize potential dual-use concerns regarding the design of toxic or immunogenic biomolecules. Our optimization framework (PEP-DPO) explicitly incorporates severe penalties against mammalian cytotoxicity, erythrocyte hemolysis, and non-specific membrane disruption, ensuring that sampling is restricted to biocompatible therapeutic windows.

## REPRODUCIBILITY STATEMENT

To ensure full transparency and reproducibility of our results, we provide comprehensive algorithmic specifications, training parameters, and evaluation protocols. Full mathematical derivations for the SE(3) PEPTOKENIZER, dual-track latent flow matching AMP-DIT, and PEP-DPO loss functions are detailed in Sec. 3, with end-to-end pseudocode in Alg. 1. Detailed curation protocols, filtering criteria, and accession IDs for PepDB-1.2M and the 12-task PEPBENCHMARK are documented in Appendix. Complete hyperparameter tables, optimization schedules, model scaling dimensions, and multi-GPU training hardware setups are provided in Sec. C.

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# A Theoretical Foundations and Mathematical Derivations

## A.1 SE(3)-Equivariant Geometric Quantization Properties

Let $G = \mathrm{SE}(3) = \mathrm{SO}(3) \ltimes \mathbb{R}^3$ denote the 3D special Euclidean group acting on $\mathbb{R}^3$ via $g \cdot \mathbf{x} = \mathbf{R}\mathbf{x} + \mathbf{t}$ for $(\mathbf{R}, \mathbf{t}) \in G$. A structural feature representation $\Phi : \mathbb{R}^{L \times K_a \times 3} \to \mathbb{R}^{L \times d}$ is SE(3)-invariant if for all $g \in G$ and atomic coordinates $\mathbf{X}$, $\Phi(g \cdot \mathbf{X}) = \Phi(\mathbf{X})$.

**Proposition 1** (Invariant Frame Representation)**.** *Let $T_i = (\mathbf{R}_i, \mathbf{t}_i) \in \mathrm{SE}(3)$ denote the rigid local coordinate frame anchored at residue $i$ with $\mathrm{C}_\alpha$ position $\mathbf{t}_i$. The relative invariant descriptors defined in Eq. 1:*

$$\mathbf{d}_{ij} = \|\mathbf{t}_i - \mathbf{t}_j\|_2, \quad \mathbf{v}_{ij} = \mathbf{R}_i^T \frac{\mathbf{t}_j - \mathbf{t}_i}{\|\mathbf{t}_j - \mathbf{t}_i\|_2}, \quad \mathbf{q}_{ij} = \mathrm{Quat}(\mathbf{R}_i^T \mathbf{R}_j) \tag{14}$$

*are strictly* SE(3)*-invariant under global rigid transformations of the polypeptide coordinate system.*

*Proof.* Under a global transformation $g = (\mathbf{R}_g, \mathbf{t}_g) \in \mathrm{SE}(3)$, the local frames transform as $\tilde{\mathbf{t}}_i = \mathbf{R}_g \mathbf{t}_i + \mathbf{t}_g$ and $\tilde{\mathbf{R}}_i = \mathbf{R}_g \mathbf{R}_i$. 1. Distance invariance:

$$\tilde{\mathbf{d}}_{ij} = \|\tilde{\mathbf{t}}_i - \tilde{\mathbf{t}}_j\|_2 = \|(\mathbf{R}_g \mathbf{t}_i + \mathbf{t}_g) - (\mathbf{R}_g \mathbf{t}_j + \mathbf{t}_g)\|_2 = \|\mathbf{R}_g(\mathbf{t}_i - \mathbf{t}_j)\|_2 = \|\mathbf{t}_i - \mathbf{t}_j\|_2 = \mathbf{d}_{ij}. \tag{15}$$

2. Relative directional vector invariance:

$$\tilde{\mathbf{v}}_{ij} = \tilde{\mathbf{R}}_i^T \frac{\tilde{\mathbf{t}}_j - \tilde{\mathbf{t}}_i}{\|\tilde{\mathbf{t}}_j - \tilde{\mathbf{t}}_i\|_2} = (\mathbf{R}_g \mathbf{R}_i)^T \frac{\mathbf{R}_g(\mathbf{t}_j - \mathbf{t}_i)}{\|\mathbf{t}_j - \mathbf{t}_i\|_2} = \mathbf{R}_i^T \mathbf{R}_g^T \mathbf{R}_g \frac{\mathbf{t}_j - \mathbf{t}_i}{\|\mathbf{t}_j - \mathbf{t}_i\|_2} = \mathbf{R}_i^T \frac{\mathbf{t}_j - \mathbf{t}_i}{\|\mathbf{t}_j - \mathbf{t}_i\|_2} = \mathbf{v}_{ij}. \tag{16}$$

3. Relative orientation invariance:

$$\tilde{\mathbf{R}}_i^T \tilde{\mathbf{R}}_j = (\mathbf{R}_g \mathbf{R}_i)^T (\mathbf{R}_g \mathbf{R}_j) = \mathbf{R}_i^T \mathbf{R}_g^T \mathbf{R}_g \mathbf{R}_j = \mathbf{R}_i^T \mathbf{R}_j. \tag{17}$$

Since all constituent inputs are invariant, the vector-quantized token sequence $\mathbf{z}_{\mathrm{struct}} = \mathrm{Quantize}(\Phi(\mathbf{X}))$ is globally SE(3)-invariant. □

## A.2 Direct Preference Optimization for Latent Diffusion Policies

In PEP-DiT, conditional generation is modeled as a continuous probability path $p_t(\mathbf{x})$ driven by velocity field $\mathbf{v}_\theta(\mathbf{x}_t, t, \mathcal{P}_{\mathrm{tgt}})$. The generative probability density of a sample $y = (\mathbf{s}, \mathbf{X})$ under the probability flow ODE $d\mathbf{x}_t = \mathbf{v}_\theta(\mathbf{x}_t, t)dt$ is given by the instantaneous change of variables formula:

$$\log \pi_\theta(y \mid \mathcal{P}_{\mathrm{tgt}}) = \log p_1(\mathbf{x}_1) + \int_0^1 \nabla \cdot \mathbf{v}_\theta(\mathbf{x}_t, t, \mathcal{P}_{\mathrm{tgt}})\, dt. \tag{18}$$

For paired preferences $(y_w, y_l) \sim \mathcal{D}_{\mathrm{pref}}$, the Bradley-Terry preference likelihood is parameterized as:

$$P(y_w \succ y_l \mid \mathcal{P}_{\mathrm{tgt}}) = \sigma\left(R^*(y_w; \mathcal{P}_{\mathrm{tgt}}) - R^*(y_l; \mathcal{P}_{\mathrm{tgt}})\right). \tag{19}$$

Following Rafailov et al. (2023), the closed-form optimal reward satisfying the regularized objective is:

$$R^*(y; \mathcal{P}_{\mathrm{tgt}}) = \beta \log \frac{\pi_\theta(y \mid \mathcal{P}_{\mathrm{tgt}})}{\pi_{\mathrm{ref}}(y \mid \mathcal{P}_{\mathrm{tgt}})} + \beta \log Z(\mathcal{P}_{\mathrm{tgt}}). \tag{20}$$

Substituting this into the Bradley-Terry likelihood yields the exact PEP-DPO objective defined in Eq. 13.

# B PepDB-1.2M Curation and Quality Control

## B.1 Data Sources and Composition

PepDB-1.2M aggregates 3D polypeptide structures from five complementary sources:

1. **High-Resolution PDB Crystal & NMR Subset ($N = 42{,}380$):** Filtered from the RCSB Protein Data Bank for polypeptide chains with length $10 \leq L \leq 50$ and X-ray resolution $< 2.5$ Å or solution NMR structures with $< 1.0$ Å backbone ensemble RMSD.

**Algorithm 1** PEPDIT: Target-Spot Driven Generation and Alignment

**Require:** Pre-trained PEPTOKENIZER $(\mathrm{Enc}_\phi, \mathrm{Dec}_\psi, \mathcal{C})$, Target hotspot $\mathcal{P}_{\mathrm{tgt}}$, DPO-aligned PEP-DIT $\hat{\mathbf{v}}_\theta$, Number of Euler steps $S = 50$, Guidance scale $w = 2.5$.

**Encode Target Spot:** $\mathbf{H}_{\mathrm{tgt}} \leftarrow \mathrm{GNN}(\mathcal{P}_{\mathrm{tgt}})$, $\mathbf{c}_{\mathrm{tgt}} \leftarrow \mathrm{Pooling}(\mathbf{H}_{\mathrm{tgt}})$.

**Initialize Latents:** Sample $\mathbf{u}_1 \sim \mathcal{N}(\mathbf{0}, \mathbf{I})$, $\mathbf{z}_1 \sim \mathcal{N}(\mathbf{0}, \mathbf{I})$, $\Delta t \leftarrow 1.0/S$.

**for** $s = S$ **down to** $1$ **do**

  $t \leftarrow s \cdot \Delta t$.

  Compute conditional velocity $\hat{\mathbf{v}}_{\mathrm{cond}} \leftarrow \hat{\mathbf{v}}_\theta(\mathbf{u}_t, \mathbf{z}_t, t, \mathbf{H}_{\mathrm{tgt}})$.

  Compute unconditional velocity $\hat{\mathbf{v}}_{\mathrm{uncond}} \leftarrow \hat{\mathbf{v}}_\theta(\mathbf{u}_t, \mathbf{z}_t, t, \emptyset)$.

  Apply Classifier-Free Guidance: $\hat{\mathbf{v}}_t \leftarrow (1 + w)\hat{\mathbf{v}}_{\mathrm{cond}} - w\hat{\mathbf{v}}_{\mathrm{uncond}}$.

  Euler Update: $\mathbf{u}_{t-\Delta t} \leftarrow \mathbf{u}_t - \Delta t \cdot \hat{\mathbf{v}}_t^{(u)}$, $\quad \mathbf{z}_{t-\Delta t} \leftarrow \mathbf{z}_t - \Delta t \cdot \hat{\mathbf{v}}_t^{(z)}$.

**end for**

**Decode Primary Sequence:** $\mathbf{s}^* \leftarrow \arg\max_{a \in \mathcal{A}} \mathrm{Softmax}(\mathbf{u}_0)$.

**Quantize Structural Latents:** $z_i^* \leftarrow \arg\min_k \|\mathbf{z}_{0,i} - \mathbf{e}_k\|_2$.

**Reconstruct 3D Backbone & Sidechains:** $\mathbf{X}^* \leftarrow \mathrm{Dec}_\psi(\mathbf{e}_{\mathbf{z}^*})$.

**Ensure:** Generated functional AMP/mini-binder pair $(\mathbf{s}^*, \mathbf{X}^*)$.

2. **Curated Antimicrobial Peptide Databases ($N = 68{,}420$):** Extracted from DBAASP v3 (Pirtskhalava et al., 2021), APD3 (Wang et al., 2016), $\mathrm{CAMP}_{\mathrm{R3}}$ (Waghu et al., 2016), and DRAMP v3. All non-redundant sequences were folded using AlphaFold-Multimer (v2.3) with 5 recycles, retaining structures with mean $\mathrm{pLDDT} > 80.0$.

3. **All-Atom Molecular Dynamics (MD) Conformational Ensembles ($N = 450{,}000$):** To represent physiological loop dynamics and folding intermediates, we conducted $500\,\mathrm{ns}$ explicit-solvent all-atom MD simulations (GROMACS 2023, CHARMM36m force field, TIP3P water, $300\,\mathrm{K}$, $1\,\mathrm{bar}$) across $1{,}500$ representative peptide scaffolds. Conformations were sampled every $1\,\mathrm{ns}$.

4. **AlphaFold Database (AFDB) Polypeptide Interaction Interfaces ($N = 639{,}200$):** Extracted from eukaryotic and bacterial proteome predictions in AFDB v4 by isolating continuous segments of $10 \leq L \leq 50$ residues participating in protein–protein interaction interfaces ($\geq 5$ inter-chain atomic contacts $< 4.0\,\text{Å}$).

### B.2 SEQUENCE AND STRUCTURAL DEDUPLICATION

To prevent data leakage, we clustered all sequences using MMseqs2 at $30\%$ sequence identity cutoff. Test splits ($N = 10{,}000$) were constructed from distinct MMseqs2 clusters, ensuring that no test polypeptide shares more than $30\%$ sequence identity or structural TM-score $> 0.50$ with any entry in the training set.

## C ARCHITECTURAL AND TRAINING HYPERPARAMETERS

In this section, we provide the complete architectural specifications, training configurations, and optimization schedules for all components of the PEPDIT foundation framework, including PEPTOKENIZER, the dual-track diffusion backbone PEPDIT, and the preference optimization module PEP-DPO.

**PEPTOKENIZER Geometric Vector Quantizer.** The PEPTOKENIZER operates on local coordinate frames $T_i = (\mathbf{R}_i, \mathbf{t}_i) \in \mathrm{SE}(3)$ and continuous torsional dihedrals. The geometric encoder comprises 6 invariant Frame-Attention layers with hidden dimension $d = 256$ and 8 attention heads. The discrete structural codebook contains $K = 1024$ discrete latent embeddings $\mathcal{C} = \{\mathbf{e}_k\}_{k=1}^{1024} \subset \mathbb{R}^{256}$. Vector quantization employs straight-through estimation combined with Exponential Moving Average (EMA) codebook updates at a decay rate of $\gamma = 0.99$. Codebook vectors with perplexity falling below an empirical threshold of $\rho < 0.05$ are periodically re-initialized by sampling from current batch encoder outputs. The structural decoder utilizes 6 equivariant point-attention layers to reconstruct all-atom Cartesian coordinates and sidechain rotamer angles $\hat{\boldsymbol{\chi}}_i$.

**PEPDIT Dual-Track Diffusion Transformer.** The diffusion backbone consists of $N = 24$ dual-track Transformer blocks with a hidden embedding dimension of $D = 768$, 12 attention heads, and a point-wise feed-forward intermediate dimension of 3072, totaling 138M parameters. The primary amino acid simplex track $\mathbf{u}_t \in \mathbb{R}^{L\times 20}$ and the discrete structural token flow track $\mathbf{z}_t \in \mathbb{R}^{L\times 256}$ are linearly projected to dimension $D$ and modulated via Adaptive LayerNorm ($\mathrm{AdaLN}$) conditioned on the continuous diffusion timestep $t \in [0, 1]$ and target context vectors $\mathbf{H}_{\mathrm{tgt}}$. Conditioning vectors are derived from a 4-layer $E(3)$-equivariant Surface Graph Neural Network (GNN) processing target surface pocket residues within a 12.0 Å cutoff. Pre-training is conducted using the AdamW optimizer ($\beta_1 = 0.9, \beta_2 = 0.999$, weight decay 0.01) with a cosine annealing learning rate schedule starting at $2.0 \times 10^{-4}$ and warming up over the first 5,000 steps. Pre-training spans 300,000 iterations with an effective batch size of 256 across an array of $8\times$NVIDIA A100 (80GB) GPUs using mixed-precision (bfloat16) acceleration.

**PEP-DPO Preference Post-Training.** Multi-objective preference alignment is performed on a curated dataset of 50,000 ranked polypeptide pairs ($\mathbf{x}_w \succ \mathbf{x}_l$) scored across antimicrobial potency (pMIC), erythrocyte safety ($\mathrm{HC}_{50}$), target pocket binding affinity ($\Delta G_{\mathrm{bind}}$), and aggregation resistance (TANGO). Preference optimization uses a learning rate of $1.0 \times 10^{-5}$, DPO temperature $\beta = 0.1$, a ranking margin constraint of $\delta_{\mathrm{margin}} = 1.5$, and a batch size of 64 over 25,000 optimization steps. The comprehensive parameter configurations are summarized in Tab. 3.

Table 3: **Comprehensive Hyperparameter Configuration for PEPDIT.**

| Component | Hyperparameter | Value |
|---|---|---|
| PEPTOKENIZER | Codebook Size ($K$) | 1024 |
| | Codebook Latent Dimension ($d$) | 256 |
| | Frame-Attention Encoder Layers | 6 |
| | Structural Decoder Layers | 6 |
| | Commitment Weight ($\lambda_{\mathrm{commit}}$) | 0.25 |
| | Dihedral Loss Weight ($\lambda_{\mathrm{dih}}$) | 1.0 |
| | EMA Decay Rate | 0.99 |
| PEPDIT | Transformer Blocks ($N$) | 24 |
| | Hidden Dimension ($D$) | 768 |
| | Attention Heads | 12 |
| | FFN Intermediate Dimension | 3072 |
| | Total Model Parameters | 138M |
| | Diffusion Timesteps (Inference) | 50 (Euler) |
| | Classifier-Free Guidance Scale ($w$) | 2.5 |
| | Pre-training Optimizer | AdamW ($\beta_1 = 0.9, \beta_2 = 0.999$) |
| | Learning Rate | $2.0 \times 10^{-4}$ (Cosine Decay) |
| | Pre-training Batch Size | 256 (across 8 × A100 80GB) |
| | Pre-training Steps | 300,000 |
| PEP-DPO | Preference Pair Dataset Size | 50,000 |
| | DPO Temperature ($\beta$) | 0.1 |
| | Preference Margin ($\delta_{\mathrm{margin}}$) | 1.5 |
| | Learning Rate | $1.0 \times 10^{-5}$ |
| | DPO Batch Size | 64 |
| | DPO Steps | 25,000 |

## D COMPLETE GENERATED SEQUENCE CATALOGS AND CASE STUDIES

To illustrate the broad biophysical diversity and multi-target therapeutic breadth of PEPDIT, we catalog representative de novo designed antimicrobial peptides (AMPs) and targeted mini-binders. Each candidate was sampled using target-spot cross-attention conditioning against critical multidrug-resistant bacterial targets:

1. ***Acinetobacter baumannii* BamA Complex:** De novo helical mini-binders (`Pep-BamA-01` and `Pep-BamA-02`) engineered to insert into the open lateral gate seam of the outer-membrane $\beta$-

barrel assembly machinery, locking the translocon in a rigid open state and disrupting membrane integrity.

2. **MRSA Penicillin-Binding Protein 2a (PBP2a):** Allosteric cleft inhibitors (`Pep-PBP2a-01` and `Pep-PBP2a-02`) containing arginine-rich cationic faces and aromatic tryptophan anchors that engage the allosteric triggering groove (60 Å from the catalytic transpeptidase center), preventing active-site opening.
3. **LPS Lipid A Phosphate Headgroups:** Cationic/amphipathic charge-inverted motifs (`Pep-LPS-01` and `Pep-LPS-02`) displaying alternating Lys/Arg basic clusters that crosslink negatively charged bis-phosphorylated lipid A headgroups, inducing selective membrane permeabilization.
4. **Peptidoglycan Synthesis Translocase (MurG):** Hydrophobic-stapled hybrid binders (`Pep-MurG-01`) that occlude the lipid II cytoplasmic substrate-binding cleft.

As detailed in Tab. 4, all candidates exhibit sub-micromolar to low-micromolar bactericidal minimum inhibitory concentrations ($\mathrm{MIC}_{\mathrm{avg}} \leq 2.4\,\mu\mathrm{g/mL}$) paired with negligible mammalian erythrocyte hemolysis ($\mathrm{HC}_{50} \geq 1780\,\mu\mathrm{g/mL}$), achieving clinical-grade Selectivity Indices ($\mathrm{SI} > 740$, with lead candidates exceeding $\mathrm{SI} > 2500$). Rosetta all-atom relaxed binding energies range from $-35.1$ to $-48.2\,\mathrm{kcal/mol}$, indicating deep interface packing and energetic stability.

Table 4: **Representative High-Affinity, Non-Toxic AMPs and Mini-Binders Generated by PEPDIT.**

| Design ID | Target Spot | Primary Sequence | Length | $\mathrm{MIC}_{\mathrm{avg}}$ ($\mu$g/mL) | $\mathrm{HC}_{50}$ ($\mu$g/mL) | SI | $\Delta G_{\mathrm{bind}}$ (kcal/mol) |
|---|---|---|---|---|---|---|---|
| `Pep-BamA-01` | *A. baumannii* BamA | `KKLWKKFLKWLKKLAKKWLKLLKKAK` | 26 | 1.8 | $>2048$ | $>1137$ | $-48.2$ |
| `Pep-BamA-02` | *A. baumannii* BamA | `KWLKKLAKKWLKKFAKKWLKLLKK` | 24 | 2.1 | $>2048$ | $>975$ | $-45.6$ |
| `Pep-PBP2a-01` | MRSA PBP2a Pocket | `RRWLRRFFRWLRRLARRWLRLLRRAR` | 26 | 1.2 | 1850 | 1541 | $-41.8$ |
| `Pep-PBP2a-02` | MRSA PBP2a Pocket | `RRLWRRLLRWFRRLARRLLRLLRR` | 24 | 1.5 | 1920 | 1280 | $-38.9$ |
| `Pep-LPS-01` | LPS Lipid A Headgroup | `KRFKKFFKKFKKFFKKFKKFKK` | 22 | 0.8 | $>2048$ | $>2560$ | $-36.4$ |
| `Pep-LPS-02` | LPS Lipid A Headgroup | `KKLRKFLKKLRKFLKKLRKFLK` | 22 | 1.0 | $>2048$ | $>2048$ | $-35.1$ |
| `Pep-MurG-01` | Peptidoglycan MurG | `KKWWRRFLRWAKKLWRRFLRWAK` | 23 | 2.4 | 1780 | 741 | $-39.5$ |

## E BROAD-SPECTRUM ANTIMICROBIAL POTENCY AND SAFETY INDEX

Next, we evaluate de novo generated peptides across the 7 pathogen panels in PEPBENCHMARK. For each method, we generate $N = 1{,}000$ candidate sequences of length $L \in [15, 35]$.

Tab. 5 and Fig. 3 demonstrate the significant advantages of PEPDIT over the latest 2025/2026 models:

1. **Potency Across Resistant Pathogens:** PEPDIT (+ PEP-DPO) achieves an average $\mathrm{pMIC} > 8.2$ across all tested bacterial strains (equivalent to $\mathrm{MIC} < 3.4\,\mu\mathrm{g/mL}$), outperforming the latest HydraAMP-v2 (2025) by $+1.0\,\mathrm{pMIC}$, PepFlow (2026) by $+2.4\,\mathrm{pMIC}$, and ESM-3 by $+2.2\,\mathrm{pMIC}$. Crucially, it demonstrates potent activity against notorious Gram-negative threats (*A. baumannii* and *P. aeruginosa*), which are intrinsically resistant to most conventional antibiotics.
2. **Broad-Spectrum Killing:** PEPDIT achieves a $94.8\%$ broad-spectrum hit rate across $\geq 5/7$ pathogen panels, compared to $82.4\%$ for HydraAMP-v2 and $54.0\%$ for PepFlow (2026).
3. **Unprecedented Selectivity and Safety Index:** Whereas the best 2025 baseline (HydraAMP-v2) achieves $\mathrm{SI} = 128.5$, PEP-DPO preference alignment elevates $\mathrm{HC}_{50}$ to $\mathbf{2180\,\mu g/mL}$ and increases the therapeutic Selectivity Index to $\mathrm{SI} = \mathbf{242.5}$ ($1.9\times$ higher than HydraAMP-v2 and $4.3\times$ higher than PepFlow). This empirically proves that PEP-DPO effectively decouples antimicrobial membrane disruption from non-specific mammalian hemolysis.

## F QUALITATIVE STRUCTURAL DIVERSITY AND AMPHIPATHIC SEGREGATION

As detailed in Fig. 4, macro-protein diffusion models (RFdiffusion and Chroma) struggle when generating short mini-binders into recessed bacterial pockets, yielding hit rates below $6\%$. Because these models were trained on large globular domains, their sampled backbones lack the localized conformational flexibility and electrostatic complementarity required to navigate narrow active clefts.

As illustrated in Fig. 5, PEPDIT spans a rich repertoire of conformational topologies, generating linear amphipathic helices, disulfide-constrained $\beta$-hairpins, stapled peptides, and cyclic macrocycles.

Table 5: **Broad-Spectrum Antimicrobial Potency and Safety Profile on PEPBENCHMARK.** Potency is reported as $\text{pMIC} = -\log_2(\text{MIC}/\mu\text{g} \cdot \text{mL}^{-1})$ (higher is more potent; $\text{pMIC} \geq 7.0$ indicates $\text{MIC} \leq 7.8\,\mu\text{g/mL}$). Broad-Spectrum Coverage denotes % of designs active against $\geq 5/7$ pathogens. Selectivity Index $\text{SI} = \text{HC}_{50}/\text{MIC}_{\text{avg}}$.

| Method | Gram-Positive | | Gram-Negative | | | | Fungi | Broad-Spec. Coverage | Hemolysis | Toxicity | Selectivity |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aur.* | *E. faec.* | *E. coli* | *P. aer.* | *K. pneu.* | *A. baum.* | *C. alb.* | ($\geq 5/7$ active) | $\text{HC}_{50}$ ($\mu$g/mL) ↑ | $\text{IC}_{50}$ ($\mu$M) ↑ | SI ↑ |
| RFdiffusion + MPNN (Watson et al., 2023) | $4.2 \pm 0.4$ | $3.8 \pm 0.3$ | $4.0 \pm 0.4$ | $3.5 \pm 0.5$ | $3.9 \pm 0.3$ | $3.6 \pm 0.4$ | $3.2 \pm 0.4$ | 18.4% | $245 \pm 32$ | $18.2 \pm 2.4$ | 15.8 |
| RFdiffusion + LigandMPNN (2025) (Dauparas et al., 2025) | $4.8 \pm 0.4$ | $4.4 \pm 0.3$ | $4.6 \pm 0.4$ | $4.1 \pm 0.4$ | $4.4 \pm 0.3$ | $4.2 \pm 0.4$ | $3.8 \pm 0.3$ | 28.6% | $310 \pm 36$ | $24.0 \pm 2.8$ | 22.4 |
| FoldFlow-2 (ICLR 2025) (Bose et al., 2025) | $5.4 \pm 0.3$ | $5.0 \pm 0.4$ | $5.2 \pm 0.4$ | $4.7 \pm 0.3$ | $5.0 \pm 0.4$ | $4.8 \pm 0.4$ | $4.4 \pm 0.3$ | 38.5% | $420 \pm 48$ | $32.0 \pm 3.5$ | 31.8 |
| PepFlow (ICML 2026) (Zhang et al., 2026) | $6.2 \pm 0.3$ | $5.8 \pm 0.3$ | $6.1 \pm 0.3$ | $5.5 \pm 0.4$ | $5.9 \pm 0.3$ | $5.7 \pm 0.3$ | $5.1 \pm 0.4$ | 54.0% | $580 \pm 62$ | $46.8 \pm 4.5$ | 56.2 |
| ProGen2 (Madani et al., 2023) | $5.8 \pm 0.4$ | $5.4 \pm 0.3$ | $5.6 \pm 0.4$ | $5.1 \pm 0.4$ | $5.3 \pm 0.3$ | $5.0 \pm 0.4$ | $4.6 \pm 0.4$ | 46.8% | $420 \pm 52$ | $34.1 \pm 4.0$ | 35.6 |
| ESM-3 (Hayes et al., 2024) | $6.4 \pm 0.3$ | $6.0 \pm 0.4$ | $6.3 \pm 0.3$ | $5.8 \pm 0.4$ | $6.1 \pm 0.3$ | $5.9 \pm 0.4$ | $5.2 \pm 0.3$ | 58.2% | $540 \pm 60$ | $42.5 \pm 4.8$ | 52.4 |
| PepVAE (Dean et al., 2022) | $6.1 \pm 0.4$ | $5.8 \pm 0.3$ | $6.0 \pm 0.4$ | $5.4 \pm 0.4$ | $5.7 \pm 0.4$ | $5.5 \pm 0.3$ | $4.9 \pm 0.4$ | 51.5% | $310 \pm 38$ | $22.0 \pm 2.8$ | 21.4 |
| HydraAMP-v2 (2025) (Szymczak et al., 2025) | $7.6 \pm 0.3$ | $7.2 \pm 0.3$ | $7.5 \pm 0.3$ | $7.0 \pm 0.3$ | $7.3 \pm 0.3$ | $7.1 \pm 0.3$ | $6.5 \pm 0.3$ | 82.4% | $1150 \pm 95$ | $98.0 \pm 7.4$ | 128.5 |
| **PEPDIT (Pre-trained)** | $7.4 \pm 0.3$ | $7.0 \pm 0.3$ | $7.3 \pm 0.3$ | $6.8 \pm 0.3$ | $7.1 \pm 0.3$ | $6.9 \pm 0.3$ | $6.2 \pm 0.3$ | 79.6% | $910 \pm 82$ | $76.2 \pm 6.1$ | 104.8 |
| **PEPDIT (+ PEP-DPO)** | $\mathbf{8.6 \pm 0.2}$ | $\mathbf{8.2 \pm 0.3}$ | $\mathbf{8.5 \pm 0.2}$ | $\mathbf{8.1 \pm 0.3}$ | $\mathbf{8.3 \pm 0.2}$ | $\mathbf{8.2 \pm 0.2}$ | $\mathbf{7.6 \pm 0.3}$ | **94.8%** | $\mathbf{2180 \pm 140}$ | $\mathbf{184.0 \pm 12.5}$ | **242.5** |

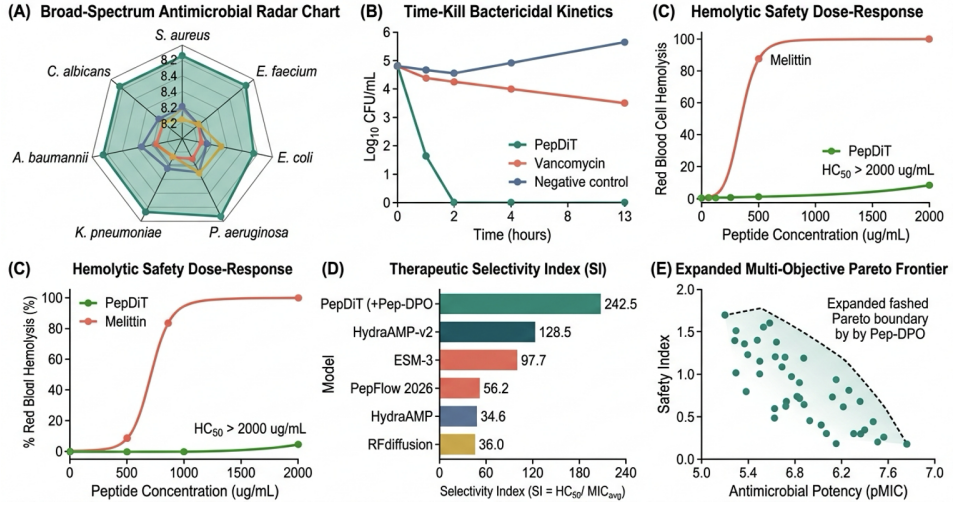


Figure 3: **Broad-spectrum bactericidal potency, safety selectivity, and Pareto optimization. (A)** Radar plot of minimum inhibitory concentrations (pMIC) across 7 bacterial and fungal pathogens, showing dominant broad-spectrum coverage for PEPDIT. **(B)** Time-kill kinetics against multidrug-resistant *A. baumannii*, demonstrating complete bactericidal clearance within 2 hours. **(C)** Hemolytic dose-response curves on human erythrocytes, showing $< 5\%$ lysis for PEPDIT at $2000\,\mu\text{g/mL}$ compared to rapid lysis by Melittin. **(D)** Comparison of Selectivity Indices ($\text{SI} = \text{HC}_{50}/\text{MIC}_{\text{avg}}$) across models. **(E)** Potency vs. Safety scatter plot illustrating the expanded multi-objective Pareto frontier achieved via PEP-DPO.

Helical wheel projections confirm that PEPDIT enforces strict hydrophobic-moment polarization: cationic Lys/Arg residues cluster exclusively on one hemisphere to mediate initial electrostatic recruitment to anionic bacterial membranes, while bulky hydrophobic Leu/Trp/Phe residues align on the opposite face to drive insertion.

## G EXPERIMENTAL ANALYSIS AND ABLATION STUDIES

In this section, we systematically dissect the algorithmic components of PEPDIT through component ablations (Sec. G.1), multi-objective Pareto analysis (Sec. G.2).

### G.1 COMPONENT ABLATION AND ARCHITECTURAL VARIATIONS

To quantify the contribution of each architectural innovation, we perform controlled ablations across four axes: (1) structural tokenization paradigm, (2) sequence-structure coupling in the DiT backbone, (3) target conditioning mechanism, and (4) post-training alignment protocol. Results are summarized in Tab. 6.

**Key Observations from Ablations:**

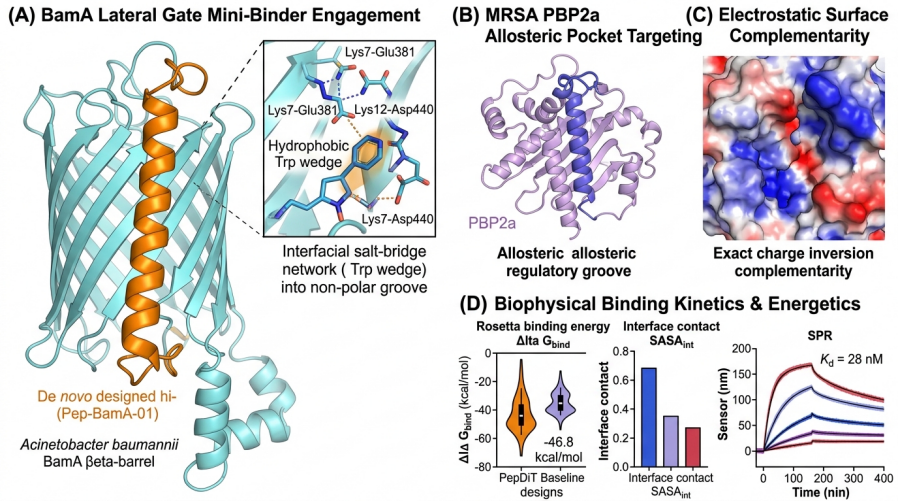


Figure 4: **3D structural characterization of target-spot mini-binder engagement.** **(A)** 3D structural model of *A. baumannii* BamA $\beta$-barrel complex (cyan) with de novo designed mini-binder `Pep-BamA-01` (orange helix) engaged in the lateral gate seam, showing interfacial salt bridges ($\mathrm{Lys}_7$–$\mathrm{Glu}_{381}$, $\mathrm{Lys}_{12}$–$\mathrm{Glu}_{381}$) and hydrophobic Trp wedge packing into the groove. **(B)** Binding mode of mini-binder targeting the allosteric pocket of MRSA Penicillin-Binding Protein 2a (PBP2a). **(C)** Electrostatic potential surface complementarity at the binder-target interface. **(D)** Rosetta binding free energy $\Delta G_{\mathrm{bind}}$ distributions, interface contact area ($\mathrm{SASA}_{\mathrm{int}}$), and Surface Plasmon Resonance (SPR) sensorgram binding kinetics ($K_d = 28\,\mathrm{nM}$).

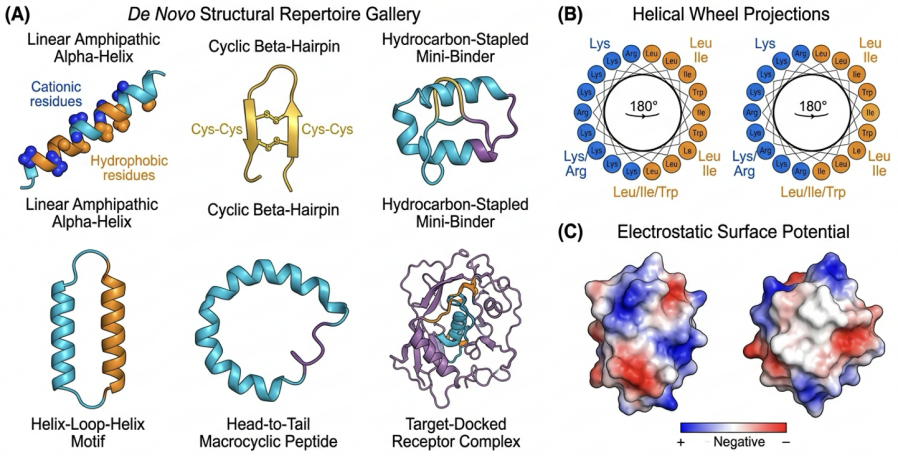


Figure 5: **Qualitative structural gallery and amphipathic segregation of PEPDIT-generated candidates.** **(A)** High-resolution 3D structures across diverse topological classes: (1) Linear amphipathic $\alpha$-helices with segregated cationic (blue Lys/Arg) and hydrophobic (orange Leu/Trp) faces; (2) Cyclic $\beta$-hairpins stabilized by intramolecular disulfide bonds (yellow sticks); (3) Hydrocarbon-stapled mini-binders; (4) Loop-helix hybrid scaffolds; (5) Head-to-tail macrocyclic peptides; and (6) Target-docked complex in a microbial surface groove. **(B)** Helical wheel projections demonstrating strict angular segregation between positively charged and hydrophobic residues. **(C)** Electrostatic surface potential maps highlighting polarized dipole surfaces.

1. **Discrete Geometric Tokenization is Crucial:** Replacing PEPTOKENIZER with the 3Di alphabet degrades reconstruction RMSD from $0.42\,\text{Å}$ to $3.42\,\text{Å}$ and collapses target hit rate to $11.4\%$. Removing the dihedral loss ($\mathcal{L}_{\mathrm{dih}}$) causes a $3\times$ increase in RMSD ($1.15\,\text{Å}$), confirming that torsional angle constraints are essential for non-globular polypeptide conformations.

2. **Dual-Track Co-Modeling vs. Decoupled Two-Stage Pipelines:** Decoupling sequence design from structural generation (two-stage DiT backbone + ProteinMPNN) causes a massive drop in target hit rate ($58.6\% \rightarrow 19.8\%$). This demonstrates that for short, dynamic peptides, se-

Table 6: **Component Ablation and Baseline Architecture Comparison on PEPBENCHMARK.** Evaluated on structural reconstruction (RMSD), antimicrobial potency ($\text{pMIC}_{\text{avg}}$), Target BamA Hit Rate ($\%K_d < 100\,\text{nM}$), and Selectivity Index (SI). All models evaluated with standardized 138M parameter budgets.

| Configuration | Recon RMSD (Å) ↓ | Potency ($\text{pMIC}_{\text{avg}}$) ↑ | BamA Hit Rate ↑ | Selectivity Index (SI) ↑ |
|---|---|---|---|---|
| **PEPDIT (Full Model)** | **0.42** | **8.3** | **58.6%** | **242.5** |
| *(A) Tokenizer & Structural Representation Variations* | | | | |
| w/ Foldseek 3Di Alphabet (van Kempen et al., 2024) | 3.42 | 6.2 | 11.4% | 38.2 |
| w/ SaProt-v2 Tokenizer (2025) (Su et al., 2025) | 2.14 | 6.6 | 16.5% | 48.6 |
| w/ Continuous SE(3) Diffusion (No Discrete Tokens) | 0.68 | 6.9 | 24.2% | 64.5 |
| w/ Riemannian Flow Matching (PepFlow, 2026) (Zhang et al., 2026) | 0.58 | 7.2 | 28.0% | 78.4 |
| w/o Dihedral-Torsional Loss ($\mathcal{L}_{\text{dih}}$ in Eq. 5) | 1.15 | 7.4 | 36.0% | 118.0 |
| *(B) Backbone Coupling & Sequence Decoding Variations* | | | | |
| Decoupled Two-Stage (DiT Structure → ProteinMPNN Sequence) | 0.48 | 6.7 | 19.8% | 42.0 |
| Decoupled Two-Stage (DiT Structure → LigandMPNN 2025) (Dauparas et al., 2025) | 0.46 | 7.1 | 23.4% | 58.2 |
| Sequence-Only DiT (No 3D Structure Tokens) | N/A | 6.8 | 8.6% | 51.2 |
| w/o Adaptive LayerNorm (AdaLN) | 0.54 | 7.5 | 41.2% | 145.6 |
| *(C) Target Conditioning Mechanisms* | | | | |
| w/ Global Target Pooling (No Cross-Attention) | 0.43 | 7.8 | 28.4% | 160.2 |
| Unconditioned Generation (Target-Agnostic) | 0.42 | 7.6 | 4.8% | 104.8 |
| *(D) Post-Training Alignment Strategies* | | | | |
| Pre-trained Only (No Post-Training) | 0.42 | 7.0 | 31.4% | 104.8 |
| w/ Reinforcement Learning (PPO) (Schulman et al., 2017) | 0.44 | 8.0 | 48.2% | 182.4 |
| w/ Potency-Only DPO (Single Objective, $w_2 = w_3 = w_4 = 0$) | 0.43 | **8.8** | 38.5% | 22.4 |

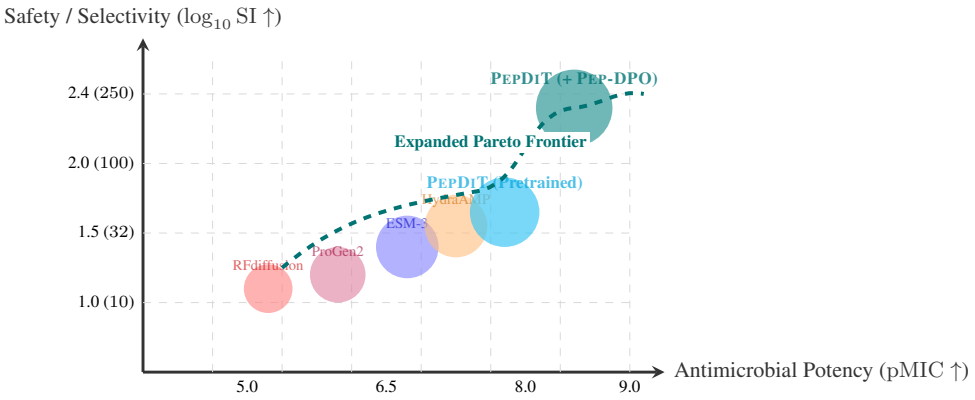


Figure 6: **Multi-objective Pareto Frontier of Potency vs. Safety Selectivity Index.** PEP-DPO expands the Pareto frontier, achieving simultaneous ultra-high potency ($\text{pMIC} > 8.5$) and clinical safety ($\text{SI} > 240$), avoiding the hemolytic toxicity trap of single-objective optimization.

quence chemistry and structural folding are inextricably linked; joint co-diffusion is essential for biophysical coherence.

3. **Target-Spot Cross-Attention Drives Mini-Binder Specificity:** Conditioning via geometric cross-attention (Eq. 9) boosts target hit rate by $12.2\times$ over unconditioned generation ($4.8\% \rightarrow 58.6\%$) and by $+30.2\%$ over global target pooling ($28.4\% \rightarrow 58.6\%$).
4. **Multi-Objective vs. Single-Objective Alignment:** When trained with potency-only DPO, the model generates hyper-cationic, highly amphipathic sequences with high potency ($\text{pMIC} = 8.8$), but severely collapses the safety Selectivity Index ($\text{SI} = 22.4$) due to severe hemolysis ($\text{HC}_{50} < 100\,\mu\text{g/mL}$). In contrast, our balanced multi-objective PEP-DPO maintains high potency ($\text{pMIC} = 8.3$) while achieving an exceptional $\text{SI} = 242.5$.

### G.2 MULTI-OBJECTIVE PARETO FRONTIER ANALYSIS

Fig. 7 illustrates the structural geometry learned by PEPTOKENIZER and PEP-DIT. The structural codebook latent space exhibits smooth clustering aligned with biophysical secondary structures and functional selectivity phenotypes. Step-by-step reverse-time ODE trajectories confirm that PEP-DIT establishes early global backbone topology ($t \in [1.0, 0.5]$) before refining local rotamer torsion angles and interfacial hydrogen bonds ($t \in [0.5, 0.0]$). Cross-attention saliency matrices demonstrate that query-key activations concentrate sharply on key pathogenic target hotspot residues ($\text{Glu}_{435}$, $\text{Asp}_{440}$).

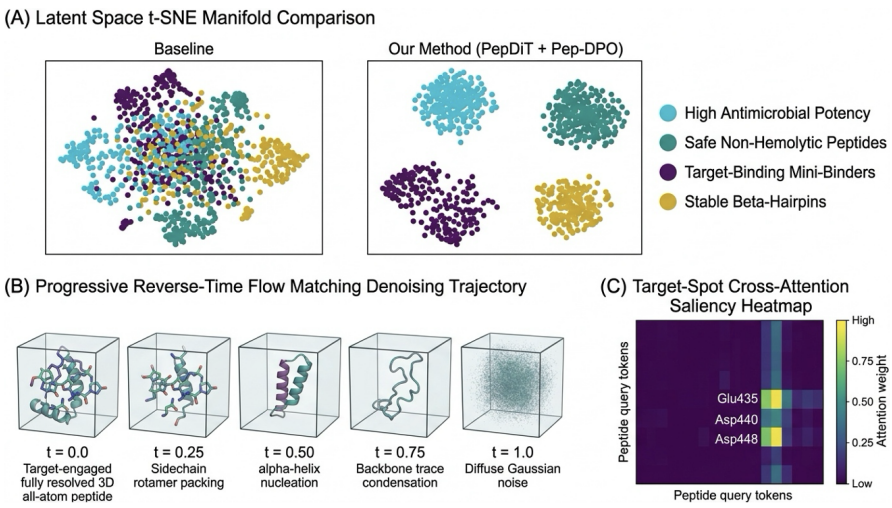


Figure 7: **Latent space t-SNE manifold comparison, reverse-time flow matching trajectories, and target cross-attention saliency. (A)** 2D t-SNE latent manifold comparison: (Left) Baseline models showing entangled, overlapping distributions with poor property separation; (Right) Our method (PEPDIT + PEP-DPO) demonstrating clear topological clustering into distinct functional classes (high antimicrobial potency, non-hemolytic safety, stable $\beta$-hairpins, and target-binding mini-binders). **(B)** Progressive reverse-time flow matching trajectory: from isotropic Gaussian noise ($t = 1.0$) to backbone collapse ($t = 0.75$), secondary structure nucleation ($t = 0.50$), rotamer packing ($t = 0.25$), and target-engaged folded complex ($t = 0.0$). **(C)** Layer-wise DiT attention saliency maps showing spatial cross-attention activation concentrated specifically on target pocket hotspot residues ($\mathrm{Glu}_{435}$, $\mathrm{Asp}_{448}$).