ProtFlow: Fast Protein Sequence Design via Flow Matching on Compressed Protein Language Model Embeddings

Zitai Kong, Yiheng Zhu, Yinlong Xu, Hanjing Zhou, Mingzhe Yin, Jialu Wu, Hongxia Xu, Chang-Yu Hsieh, Tingjun Hou, Jian Wu

Zhejiang University

{kongzitai, zhuyiheng2020, 22321336, jialuwu, Einstein, kimhsieh, tingjunhou, wujian2000}@zju.edu.cn, {zhj85393, mzyin256}@gmail.com

Abstract

The design of protein sequences with desired functionalities is a fundamental task in protein engineering. Deep generative methods, such as autoregressive models and diffusion models, have greatly accelerated the discovery of novel protein sequences. However, these methods mainly focus on local or shallow residual semantics and suffer from low inference efficiency, large modeling space and high training cost. To address these challenges, we introduce PROTFLOW, a fast flow matching-based protein sequence design framework that operates on embeddings derived from semantically meaningful latent space of protein language models. By compressing and smoothing the latent space, Prot-Flow enhances performance while training on limited computational resources. Leveraging reflow techniques, ProtFlow enables high-quality singlestep sequence generation. Additionally, we develop a joint design pipeline for the design scene of multichain proteins. We evaluate ProtFlow across diverse protein design tasks, including general peptides and long-chain proteins, antimicrobial peptides, and antibodies. Experimental results demonstrate that ProtFlow outperforms taskspecific methods in these applications, underscoring its potential and broad applicability in computational protein sequence design and analysis.

1 Introduction

Proteins are fundamental biomolecules that play crucial roles in organisms, serving as structural components of cells and facilitating various essential biological processes. The design of artificial proteins with desired functionalities has become a cornerstone of modern bioengineering research [Huang *et al.*, 2016]. However, the vast exploration space of possible protein sequences presents a significant challenge, and traditional biochemical approaches for discovering novel proteins are both time-intensive and expensive. Recently, the deployment of deep generative models has revolutionized protein design by enabling intelligent exploration of the biochemical landscape, offering a more efficient and cost-effective alternative [Zhu *et al.*, 2024a].

Given the similarities between protein sequences and natural language, advanced natural language processing (NLP) techniques, particularly autoregressive (AR) models, have been adapted for protein sequence design [Ruffolo and Madani, 2024]. However, due to fundamental differences between protein sequences and natural language expressions, the unidirectional generation nature of AR methods may not always be optimal for protein design. Since nonautoregressive (NAR) approaches, predominantly diffusion models [Ho et al., 2020], generate the entire sequence rather than predicting the next element step by step, they are better suited for modeling the long-range dependencies and complex amino acid interactions in proteins [Wang et al., 2024]. Despite their advantages, diffusion models face several challenges, including prolonged generation times, high computational costs, and sensitivity to hyperparameters [Dao et al., 2023; Hu et al., 2024]. Although score-based diffusion methods have enhanced training efficiency, their reliance on a limited set of sampling probability paths necessitates specialized techniques, which still struggle to completely avoid suboptimal outcomes [Eijkelboom et al., 2024].

Flow Matching (FM) [Lipman *et al.*, 2022] provides a more general and deterministic framework that directly learns the vector field guiding the optimal probability path from the prior noise distribution to the target data distribution, all in a simulation-free manner. FM achieves high-quality generation more efficiently by requiring significantly fewer ODE-solving steps than diffusion models. In spite of this efficiency, the optimal single-step generation can be further achieved by the Reflow technique [Liu *et al.*, 2022]. While FM has been successfully applied in various domains, including protein structure prediction [Yim *et al.*, 2024], its potential for protein sequence design remains largely unexplored. A major challenge lies in adapting continuous FM models to accommodate the inherently discrete nature of protein sequences.

Inspired by latent diffusion models [Rombach et al., 2022], we propose embedding discrete protein sequences into a latent space optimized for FM. Drawing inspiration from the transformative impact of large-scale language models in NLP [Brown, 2020], Protein Language Models (pLMs), trained on evolutionary-scale protein sequences, have demonstrated significant potential for protein design [Lin et al., 2022]. Rather than developing an encoder from scratch, we leverage pLMs as encoders to operate within their semanti-

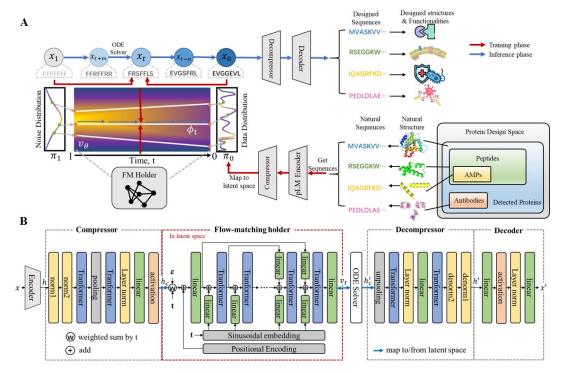


Figure 1: Overview of ProtFlow for protein sequence design. (A) Left: the visualization of the mathematical working flow of ProtFlow, including the training and inference phases. Right: relationships of different protein groups; (B) the architecture schematic of each components of ProtFlow. The FM holder is trained with other components frozen. In the training phase, the sampled sequence x is mapped to the latent space as h_c by the pLM encoder and compressor; the FM holder learns the FM vector field v_t . In the inference phase, the ODE solver starts from a sampled random noise ϵ and starting time point t, iteratively solves the h'_c with v_t represented by the FM holder, and maps back to x' by the decompressor and decoder.

cally meaningful latent spaces, thereby enhancing both controllability and flexibility [Hu et al., 2024]. However, pLM embeddings often suffer from excessively high dimensionality and massive activation issues [Valeriani et al., 2024]. To address these challenges, we redesign the pLM latent space by introducing a compressor that produces a more compact and smoothed latent representation.

Building on these insights, we introduce **ProtFlow**, a fast flow matching-based model for *de novo* protein sequence generation on the compressed and smoothed pLM embedding space, which even supports 1-step generation with reflow. We also comprehensively evaluate the performance of the model in various practical protein design scenarios. We highlight our main contributions as follows:

- We introduce ProtFlow, the first flow matching-based generative model for protein sequence design to the best of our knowledge, which supports fast designs.
- We redesign the latent space of pLMs, achieving a 16-fold embedding compression, and experimentally demonstrate the improvements resulting from this optimized latent space.
- We develop a multichain joint design pipeline and showcase ProtFlow's capability to generate structurally plausible, reliable, and natural protein sequences across various design tasks, including general peptides, long-chain proteins, antimicrobial peptides (AMPs), and antibodies,

consistently outperforming existing methods.

2 Related Work

De novo protein sequence design focuses on constructing protein sequences that belong to biologically active groups, such as enzymes [Yeh et al., 2023], antibodies [Frey et al., 2023; Mahajan et al., 2023], and peptides [Chen et al., 2024] from scratch. Deep generative models have proven effective in designing high-quality de novo protein sequences. Prominent methods include protein language models [Lin et al., 2022] and discrete diffusion models [Alamdari et al., 2023; Zhu et al., 2024b]. By encoding proteins into a latent space, advanced continuous diffusion models can be effectively applied to this task [Meshchaninov et al., 2024]. Our work leverages this continuous approach to delve into the semantically rich latent protein space.

Diffusion models [Ho *et al.*, 2020] can generate meaning-ful output by gradually denoising samples from prior noise distributions. To improve efficiency, the score-matching paradigm [Song *et al.*, 2020b] is introduced. Diffusion models have become a prominent framework in generative modeling, achieving notable success across various domains such as image synthesis [Dhariwal and Nichol, 2021], text generation [Li *et al.*, 2022], and molecular modeling [Hoogeboom *et al.*, 2022]. Despite their successes, diffusion models often encounter challenges like high computational de-

mands, prolonged generation times, and sub-optimal probability paths [Song et al., 2020a; Lu et al., 2022].

Flow matching models, proposed as an efficient, simulation-free method to train Continuous Normalizing Flows (CNFs), eliminate the need for explicit knowledge of the marginal vector field [Lipman et al., 2022]. Despite these advancements, conventional solvers still require extensive function evaluations [Dao et al., 2023]. To address these challenges, several techniques have been developed to improve performance. Rectified Flows (RFs) [Liu et al., 2022] employ straight-line simulations of probability paths, simplifying training and accelerating sampling by avoiding complex ODE solvers while preventing the collapse of word embeddings. Conditional Flow Matching (CFM)[Tong et al., 2023] learns the vector field conditioned on individual data points and incorporates Optimal Transport to ensure optimal probability paths, enhancing training efficiency and reducing inference time. While flow-based methods have shown promising results in the design of biological molecules, including protein structures [Yim et al., 2024], their application to protein sequence design remains largely unexplored. To bridge this gap, we introduce the first FM-based generative model specifically tailored for protein sequence design.

3 Background

3.1 Problem formulation

Typically, a protein can be represented as a sequence of amino acids $x = [x_1, x_2, \ldots, x_L]$ of length L, where each amino acid x_i is selected from a vocabulary $\mathcal V$ that includes the 20 standard amino acids. Deep generative models for *de novo* protein sequence design are defined to learn the data distribution p(x) and to sample novel and plausible protein sequences x from this distribution.

3.2 Preliminaries

Inspired by score-based generation models [Song *et al.*, 2020b], we can define the mapping between samples from the data distribution $x_0 \sim \pi_0$ and the prior distribution $x_1 \sim \pi_1$ as an ordinary differential equation

$$d\phi_t = v_\theta(\phi_t, t)dt \tag{1}$$

where θ means the vector field $v:[0,1]\times R^d\to R^d$ can be learned with a neural network. This vector field defines a unique time-dependent flow $\phi:[0,1]\times R^d\to R^d$.

The marginals are Gaussian conditioned on some random variable z, which are assigned with concrete meanings later.

$$p_t(x_t) = \mathbb{E}_{z \sim \mathcal{N}(\mu_t, \sigma_t)} p_t(x|z) = \mathcal{N}(x|\mu_t(z), \sigma_t^2(z))$$
 (2)

One of the simplest flow ϕ that can generate this probability path p_t is

$$\phi_{t,z}(x) = \mu_t(z) + \sigma_t(z)x \tag{3}$$

Since the vector field u_t generates the flow, we can learn the flow that matches p_t by using gradient descent in regression against the target vector field, which is called **Flow Matching** [Lipman $et\ al.$, 2022]. That is, We can regress u_t with the $Flow\ Matching\ Objective$

$$\mathcal{L}_{FM} = \mathbb{E}_{t, n_t(x)} \| v_{\theta}(x, t) - u_t(x) \|_2^2 \tag{4}$$

The unique conditional vector field can be written as

$$u_t(x|z) = \frac{\sigma'_t(z)}{\sigma'_t(z)}(x - \mu_t(z)) + \mu'_t(z)$$
 (5)

One can construct the marginal vector field $u_t(x)$ using the conditional vector field $u_t(x|z)$

$$u_t(x) = \mathbb{E}_{q(z)} \frac{u_t(x|z)p_t(x|z)}{p_t(x)}$$
(6)

Since \mathcal{L}_{FM} in intractable due to the marginalization shown in Equation 6, people introduce an equivalent tractable *Conitional Flow Matching (CFM) Objective*

$$\mathcal{L}_{CFM} = \mathbb{E}_{t,q(z),p_t(x|z)} \|v_{\theta}(x,t) - u_t(x|z)\|_2^2$$
 (7)

To reduce the complexity of the flow and accelerate the sampling process, **Rectified Flows (RFs)** [Liu *et al.*, 2022] define the probability paths as straight lines $\mu_t(z) = tx_1 + (1-t)x_0$, which further simplify the method into the form

$$q(z) = \pi_0(x_0)\pi_1(x_1) \tag{8}$$

$$p_t(x|z) = tx_1 + (1-t)x_0 (9)$$

$$u_t(x|z) = x_1 - x_0 (10)$$

where z is defined as a tuple of random variables (x_0, x_1) , representing samples from data and noise distributions.

Although rectified flows reduce the transport cost, there are still curves and crossings in the probability paths. By using the **Reflow** technique, where the corresponding pairs (z_0, z_1) with $z_0 \sim \mu_0(z)$ and $z_1 \sim \mu_1(z)$ from the learned rectified flows are sampled and replaced (x_0, x_1) to run new rectified flows, the probability paths can be made nearly straight with further reduced transport cost. In this case, good results can be sampled with even a single ODE solving step.

4 Methods

As depicted in Figure 1, we design protein sequences with rectified flow-based methods in the redesigned ESM-2 latent space with compression and smoothing. We further accelerate the generation with reflow. Detailed parameter and training settings can be found in Appendix A and algorithms can be found in Appendix B.

4.1 Design with pLM Latent Space

To leverage the semantically meaningful latent space of pLM [Hu et al., 2024], which is inherently more suitable for continuous FM-based methods, we utilize the pre-trained pLM, ESM-2 [Lin et al., 2022], as the encoder and fine-tune a corresponding decoder. ESM-2 is one of the most advanced pLMs, trained on the large-scale standard protein sequence database Uniref50 [Suzek et al., 2015]. The encoder maps a protein $x = [x_1, x_2, \dots, x_L] \in \mathcal{V}^L$ into a continuous embedding $h = [h_1, h_2, \dots, h_L] \in \mathcal{R}^{L \times D}$, while the decoder reconstructs the sequence as $x' = [x'_1, x'_2, \dots, x'_L] \in \mathcal{V}^L$. To adapt the pre-trained language model to specific tasks and improve reconstruction accuracy, the decoder is trained independently using a cross-entropy loss between the tokenized x and x'. To investigate the impact of pLM model quality, we experiment with two variants of ESM-2 with different parameter scales (8M and 35M). To standardize the length Lof all protein sequences, we add padding tokens to the ends of shorter proteins, which will implicitly encode the length information in the latent embedding.

4.2 Latent Space Redesign

The ESM-2 embeddings have unnecessarily large dimensions and the massive activation problem [Valeriani et al., 2024], necessitating the redesign of a smoother and more compact latent space. To enhance space efficiency, we introduce a dimensional compressor-decompressor pair positioned between the encoder and decoder. This mechanism compresses the original continuous embedding $h \in \mathcal{R}^{L \times D}$ to $h_c \in \mathcal{R}^{L \times D/c}$ with a compression ratio c and subsequently reconstructs it back to $h' \in \mathcal{R}^{L \times D}$. To adjust the data scale and smooth the data, we add preprocessing operations before the compressor. Since our FM model operates on a standard Gaussian distribution, we apply z-score normalization to scale the output embeddings of ESM-2. The massive activation problem introduces outliers with excessively small variances, which affects the proper convergence. To fix this, we first truncate the z-score normalized embeddings using a saturation function and employ a min-max normalization to smooth the truncated latent space. The corresponding reverse operations are added after the decompressor. With the other parts frozen, the compressor-decompressor pair is trained using an MSE reconstruction objective between h and h'.

4.3 Fast Generation with Flow Matching

1-Rectified Flow Generation

To address the long generation time and suboptimal probability path issues inherent in diffusion-based methods, we adapted the Rectified Flow-based [Liu *et al.*, 2022] method for protein sequence design. Due to its straight and optimal probability paths, our model can theoretically generate good results with fewer ODE-solving steps.

During training, we randomly sample the sequence x, standard Gaussian noise ϵ and time step t within the time range [0,1]. The sequence will be encoded and compressed to h_c .

$$q(z) = \pi_0(h_c)\pi_1(\epsilon) \tag{11}$$

Then we calculate $p_t(x|z)$ and the vector field of Rectified Flow $u_t(x|z)$ with

$$p_t(x|z) = t\epsilon + (1-t)h_c, t \sim \text{Uniform}(0,1)$$
 (12)

$$u_t(x|z) = \epsilon - h_c \tag{13}$$

Finally the model predicts the vector field $v_{\theta}(h_c, t)$ and trained with the loss function in Equation 7.

During inference, we begin by sampling a random standard Gaussian noise ϵ and iteratively solve the ODE within the learned vector field using an ODE solver. The resulting embedding h_c' is then decompressed using the decompressor and subsequently decoded back into a protein sequence. For 1-Rectified Flow, we employ the dopri5 solver and for the reflow, we use the Euler solver. The optimal number of ODE-solving steps is determined based on specific tasks, with the step range set between [1,100]. Although the length of protein sequences is implicitly captured in the latent embedding through the trailing padding tokens, we additionally sample the sequence length from the empirical distribution observed in the validation dataset and apply attention masks to ensure the adequate distribution of generated sequence lengths.

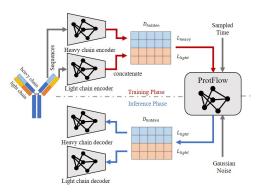


Figure 2: Joint Design of Antibodies. Heavy chains and light chains are utilized to finetune ESM-2 decoders respectively. The embeddings are concatenated before fed into ProtFlow. D_{hidden} is the ESM-2 hidden dimension; L_{heavy} and L_{light} are the maximum lengths of the heavy and light chains.

One-step Generation with Reflow

To further accelerate the generation, we employ the reflow technique to construct 2-Rectified Flow, which theoretically enables high-quality one-step generation [Liu *et al.*, 2022]. We sample from the start and end point of the ODE solver for the inference of trained 1-Rectified Flow models. The obtained paired noises and data (z_0, z_1) will replace (ϵ, h_c) in Equation 11- 13 for finetuning.

4.4 Multichain Joint Design Pipeline

For multichain proteins, such as antibodies, significant interdependence often exists among individual chains. Designing each chain independently may compromise the structural integrity of the overall protein. Therefore, we developed a pipeline for the joint design of multichain proteins. Taking antibodies as an example, as shown in Figure 2, we fine-tune two separate ESM-2 models to encode and decode the heavy chain and the light chain, and concatenate the embeddings to be processed simultaneously by ProtFlow as a whole. During the inference phase, the generated embeddings will be split and independently converted back to protein sequences.

4.5 Model Architecture

For the decoder, we employ the same architecture as the ESM-2 LMHead, which consists of two linear layers, an activation layer, and a layer norm.

The compressor consists of two dimension normalization layers, two transformer layers with a pooling layer between them, a down-projection block with a layer norm and a linear layer, and an additional activation layer to smooth the continuous embeddings. The decompressor consists of an unpooling layer, two transformer layers with an up-projection block with a layer norm and a linear layer between them, and corresponding denormalization layers.

The flow-matching holder is constructed as a 12-layer Transformer model. Time information is integrated into the model via a linear projection, and added before each Transformer block. Inspired by the U-Vit architecture [Bao *et*

al., 2023], and following insights from diffusion-based studies [Meshchaninov et al., 2024], we utilize long skip connections to enhance the performance and facilitate more effective learning dynamics.

5 Experiments

In this section, we first determine the optimal compression ratio c, and evaluate the model in the general peptide and long-chain protein generation task. Following the impressive results in general tasks, we apply ProtFlow to design a practical functional protein, antimicrobial peptides. Finally, we evaluate the model on a more challenging task, the antibody design, which contains multiple sequences and more complex structures. Please refer to Appendix C for detailed experiment settings.

5.1 Optimal Compression Ratio

To ensure the generalizability, we first determine the optimal compression ratio c on general design tasks. We evaluate a range of compression ratios [1,2,4,8,16,32] by assessing the reconstruction accuracy and FPD. The results, summarized in Table 1, demonstrate that compression does not significantly impact reconstruction accuracy. As the embedding dimensionality is progressively reduced up to a ratio of 16, the FPD values improve in both cases, indicating an enhanced capability for distribution learning. Beyond this threshold, additional compression may introduce potential trade-offs between efficiency and representation fidelity.

Table 1: Reconstruction accuracy and FPD of each Compression Ratio (c) on the general peptide and long-chain protein tasks.

Ratio (c)	Uniprot p		SwissProt		
	Acc(%)↑	FPD↓	Acc(%)↑	FPD↓	
1	99.93	0.48	99.98	2.09	
2	99.96	0.44	99.88	1.59	
4	99.97	0.42	99.89	1.35	
8	99.96	0.41	99.87	1.23	
16	99.96	0.36	99.91	1.13	
32	99.98	0.43	99.91	1.26	

5.2 General Peptide and Protein Design

This section involves the general protein design cases, including short-chain proteins, i.e. peptides, and long-chain proteins. In these general design cases, we develop ProtFlow and analyze the impacts of each technique of our methods. Experiments demonstrate its versatility and outstanding performance, laying a solid foundation for the subsequent application of ProtFlow in functional proteins.

Data For general peptides, we collected peptides with lengths of 2 to 50 from UniProt [Consortium, 2019]. After removing sequences with uncommon amino acids, we ended up with 2.6M peptides. For general long proteins, we use **SwissProt**, a high-quality, manually annotated subset of UniProt. After filtering out sequences shorter than 128 and trimming sequences longer than 254, and removing sequences with uncommon amino acids, we get a total number of 470k proteins.

Baseline We include the Generative Adversarial Network (GAN) method ProteinGAN [Repecka *et al.*, 2021], the discrete diffusion method EvoDiff-OADM with ByteNet-CNN backbone and Transformer backbone [Alamdari *et al.*, 2023], the continuous diffusion method DiMA [Meshchaninov *et al.*, 2024], and the discrete FM methods Dirichlet Flow Matching [Stark *et al.*, 2024]. All baseline models are set with the same or similar number of parameters and use a standard character-level tokenization scheme.

Metrics We follow the metrics settings of [Meshchaninov et al., 2024]. To evaluate sequence quality, we use ESM-2 pppl. To evaluate structural foldability and quality, we use OmegaFold [Wu et al., 2022] pLDDT and ESM-IF scPerplexity. To evaluate naturalness, we use TM-Score against SwissProt. To reflect the ability to capture the data distribution, we use Frechet ProtT5 Distance (FPD), Maximum mean discrepancy (MMD) and 1-Wasserstein optimal transport (OT). For the generation speed, we use the Number of Function Evaluations (NFE).

Results The results are presented in Table 2. We highlight our main findings as follows:

- (1) ProtFlow generates high-quality sequences with leading distributions. We investigate the quality of the sequences ProtFlow generates leveraging ESM-2 Perplexity and ESM-IF scPerplexity. ESM-2 Perplexity reflects how well a given sequence aligns with the patterns ESM-2 has learned from its training data, while ESM-IF scPerplexity estimates the quality and reliability on the structural level. ProtFlow achieves the best perplexity results better than the dataset, revealing that it is capable of generating protein sequences with high reliability and good-alignment with natural protein patterns. FPD, MMD, and OT measure the distributional similarity between the generated sequences and the training data. ProtFlow outperforms all baselines. This indicates that ProtFlow is a good data distribution learner. Notably, when the model gains better distribution values, its perplexities may exhibit slight degradation. This might indicate the trade-off between the computing reliability and the residue-level diversity of protein sequences.
- (2) ProtFlow generates sequences with foldable and natural predicted structures. The predicted local distance difference test (pLDDT) score is the most authoritative measurement of the structural plausibility of protein sequences. Typically, a protein with a pLDDT score greater than 70 is considered to have high structural confidence. After predicting the structure with OmegaFold, we compare the structural similarity against known structures in SwissProt PDB with TM-Score. ProtFlow generates protein sequences with the highest pLDDT score and TM-Score, which are comparable to or even better than the datasets for the general protein design tasks. This demonstrates that ProtFlow generates sequences with high foldability and structural naturalness.
- (3) Flow matching leads fast and optimal generation. One of the most significant advantages of FM is its fast generation speed. Unlike diffusion models, which require hundreds or even thousands of steps, Dirichlet FM achieves comparable results with only 50 steps, making it twice as efficient

Table 2: Performance com	parison between	ProtFlow and b	aseline models on	UniProt Peptides and	d SwissProt datasets.

	Model	pLDDT (†)	ESM-2 pppl (↓)	scPerplexity (↓)	TM-score (†)	FPD (↓)	MMD (↓)	OT (↓)	NFE (↓)
	Dataset	71.35	12.58	12.31	0.57	0.23	0.000	1.74	nan
	Random sequences	62.89	21.71	20.40	0.44	3.66	0.084	5.75	nan
iProt Peptides	ProteinGAN EvoDiff-OADM EvoDiff-OADM w/ Transformer DiMA Dirichlet FM	67.84 64.99 63.78 70.95 67.19	15.14 14.26 17.76 13.72 14.13	13.89 13.61 14.75 14.12 13.59	0.54 0.50 0.52 0.54 0.53	1.51 0.60 1.24 0.87 0.50	0.034 0.014 0.051 0.021 0.014	2.78 2.29 2.72 2.57 2.10	nan 1000 1000 100 100 50
UniPr	ProtFlow w/ ESM2 8M	71.14	12.89	13.05	0.56	0.53	0.010	2.07	25
	ProtFlow w/ ESM-2 35M	70.54	12.96	12.83	0.56	0.46	0.009	1.99	25
	ProtFlow w/ ESM-2 35M & compression	71.93	12.29	12.14	0.59	0.36	0.004	1.86	25
	ProtFlow reflow w/ ESM-2 35M & compression	72.86	12.16	11.79	0.60	0.45	0.006	1.97	1
Į,	Dataset	79.36	9.88	5.21	0.80	0.27	0.002	1.37	nan
	Random sequences	26.31	21.17	14.84	0.33	3.07	0.205	3.88	nan
SwissPr	proteinGAN	32.77	16.12	12.36	0.24	3.08	0.176	4.02	nan
	EvoDiff-OADM	39.36	15.97	10.76	0.2	2.17	0.112	3.50	1000
	DiMA	77.56	11.46	6.54	0.42	1.58	0.104	2.25	100
	Dirichlet FM	53.33	12.88	9.35	0.37	1.38	0.073	2.56	50
	ProtFlow	79.65	8.77	4.24	0.47	1.13	0.059	2.35	25

as DiMA. Leveraging the nearly linear probability path of 1-RF, ProtFlow achieves excellent results with just 25 steps, achieving a fourfold speed improvement over DiMA. Reflow further straightens the probability path, enabling even single-step generation using the simplest Euler ODE solver. Although fine-tuning based on imprecise 1-RF probability path sampling may slightly compromise distributional performance, reflow still outperforms all other methods. Moreover, by learning the optimal probability path, FM ensures excellent generation quality while maintaining fast generation speed. The most direct manifestation of this is the superiority of FM-based methods in distributional metrics. Notably, both Dirichlet FM and ProtFlow, especially the model using the same ESM2-8M with DiMA, excel beyond the diffusion models, EvoDiff and DiMA, which operate in discrete and continuous spaces, respectively. Specifically, while DiMA achieves impressive scores in the initial metrics, it performs less favorably in terms of distribution metrics compared to both flow-based methods. This consistency underscores the superior performance of FM methods, by learning and modeling optimal probability paths, excel particularly in capturing accurate data distributions.

(4) Latent space redesign improves FM effects. As a method operating on the continuous latent space built by the pLM ESM-2, ProtFlow outperforms Dirichlet FM, which directly operates on discrete sequences; Another latent method, DiMA, also achieves better scores on foldability, reliability and naturalness than discrete methods, EvoDiff and Dirichlet FM. This advantage partly reflects the benefits of working within a more condensed and semantically rich latent space. In Table 1, as the dimensionality is progressively compressed, the model's ability to learn the data distribution improves until a 16-fold compression, where excessive compression likely leads to the loss of possible probability path information, reducing the model's performance. Notably, performing FM in the compressed latent space allows the model to outperform the datasets. This indicates that latent space compression not only makes the model more compact in terms of spatial representation, but also enables more streamlined information, which further facilitates the learning of optimal probability paths through FM.

5.3 Antimicrobial Peptide Design

Antimicrobial peptides (AMPs) are a class of functional proteins with antimicrobial properties that play a crucial role in the innate immune response of mammals against invasive bacterial infections. Currently, researchers in the biochemical field are focused on developing novel and robust antimicrobial peptides, a process that generative models can assist in and accelerate. The experiments demonstrated that ProtFlow is capable of creating high-quality, novel, diverse AMPs, highlighting its significant potential to design proteins with other desired attributes for practical applications.

Data Following AMP-Diffusion [Chen *et al.*, 2024], we collected AMPs from databases dbAMP [Jhong *et al.*, 2019], AMP Scanner [Veltri *et al.*, 2018], and DRAMP [Kang *et al.*, 2019]. After filtering out sequences with length greater than 40 and containing uncommon amino acids, expanding by HydrAMP [Szymczak *et al.*, 2023] and removing duplicate sequences, 195k AMPs are left for training.

Baseline We utilize baselines from AMP-Diffusion, including two advanced conditional VAE methods HydrAMP [Szymczak *et al.*, 2023] and PepCVAE [Das *et al.*, 2018], the GAN method AMPGAN [Van Oort *et al.*, 2021], and the diffusion method AMP-Diffusion.

Metrics We also follow the metrics settings of AMP-Diffusion. To evaluate the sequence quality of the generated proteins, we use **ESM-2 pppl**. To evaluate the diversity and similarity of the generated proteins, **Shannon Entropy** and 6-mers **Jaccard Similarity** (JS-6) are utilized. Finally we use an external classifier of HydrAMP to calculate the proportion of generated peptides that can be classied as AMPs, P_{amp} and anti-E. coli, P_{mic} .

Results Table 3 delineates the performance of ProtFlow in comparison to other relevant models. We find that ProtFlow outperforms other AMP-specific methods. The lowest ESM-2 perplexity demonstrates that ProtFlow can generate highly reliable peptides. Shannon entropy serves as a measure of

Table 3: Performance on AMP design of ProtFlow and baseline methods on the AMP dataset. †: benchmarked results are quoted from [Chen et al., 2024].

Model	Perplexity(\downarrow)	Entropy(†)	JS-6(†)	$P_{\rm amp}(\uparrow)$	$P_{\mathrm{mic}}(\uparrow)$
Train/Real AMPs†	16.23 ± 3.80	3.23 ± 0.40	-	-	-
AMPGAN†	17.70 ± 4.08	2.90 ± 0.61	0.016	0.54	0.32
PepCVAE†	19.82 ± 3.83	3.12 ± 0.36	0.020	0.41	0.20
HydrAMP†	17.27 ± 6.02	2.82 ± 0.48	0.014	0.77	0.49
AMP-Diffusion†	12.84 ± 4.53	3.17 ± 0.56	0.028	0.81	0.50
ProtFlow	$\textbf{12.24} \pm \textbf{3.26}$	$\textbf{3.19} \pm \textbf{0.55}$	0.080	0.84	0.63

the sequence's uncertainty or randomness, reflecting its information content and complexity. ProtFlow gains the highest entropy, indicating that it can generate the most diverse peptide sequences. Meanwhile, ProtFlow receives a remarkably higher 6-mer JS score, indicating its ability to learn complex sequence motifs relevant to AMP design.

For the antimicrobial property classification test, we take a threshold for P_{amp} as 0.8, which can be a good likelihood of being antimicrobial. Since the MIC property is a subset of the antimicrobial property, and the peptides experimentally measured MIC to train the HydrAMP classifier is limited [Szymczak $et\ al.$, 2023], a threshold for P_{mic} set as 0.5 is enough to show the peptide activity potential against $E.\ coli.$ ProtFlow generates the notably highest proportion of peptides exceeding both thresholds, meaning ProtFlow can generate peptides not only being classified as AMPs, but also exhibiting effective activity on specific pathogenic bacteria.

5.4 Antibody Design

Antibodies are large Y-shaped proteins composed of heavy and light chains with important immune functions. The design of novel antibodies has long been a critical focus in bioengineering and medicine. However, given the complexity of antibodies, designing them requires the simultaneous generation of both the heavy and light chains. Leveraging our multichain joint design pipeline, ProtFlow is able to operate on this more complicated task. Through antibody design, we have demonstrated that ProtFlow is capable of designing large, complex proteins and can collaboratively design multiple related proteins. This further expands the potential of ProtFlow for practical application. We follow the benchmark settings of L-WJS [Mahajan *et al.*, 2023].

Data We collected 1.4M pairs of antibodies (containing heavy and light chains) from the Observed Antibody Space (OAS) database [Olsen *et al.*, 2022]. Sequences were clustered at 95% sequence identity with 80% coverage using MMseqs2 [Steinegger and Söding, 2017]. Sequences with uncommon amino acids, heavy chain longer than 149 and light chain longer than 148 are filtered out.

Baseline We use baselines including the language models GPT3.5, IgLM [Shuai *et al.*, 2021] and ESM-2 [Lin *et al.*, 2023], an energy-based method DEEN [Saremi *et al.*, 2018], and the diffusion methods dWJS [Frey *et al.*, 2023] and L-WJS [Mahajan *et al.*, 2023].

Metrics To reflect the learning quality of the training data distribution on the physicochemical property scale, we use the Wasserstein Distance $W_{property}$ between the sampled sequences and the reference distribution on 15 biological properties. To evaluate the novelty, we utilize the proportion of unique sequences (Uniqueness). To evaluate sequence diversity, we use the average edit distance of sampled sequences from the reference distribution, E_{dist} , and the average edit distance within the generated sequences, IntDiv.

Results Table 4 exhibits the performance of ProtFlow in comparison to other relevant models. Notably, ESM-2 achieves the highest E_{dist} and IntDiv. However, as mentioned in dWJS [Frey $et\ al.$, 2023], ESM-2 does not generate antibody-like sequences, making these leading scores meaningless. ProtFlow receives the lowest $W_{property}$, representing its promising learning ability of the biophysical property distributions of the paired OAS. Most of the models show good Uniqueness generation. ProtFlow gains the second best Int-Div and E_{dist} score values, which are still competitive. It might reflect the trade-off between the distribution learning and novelty together with diversity.

Table 4: Performance on antibody design of ProtFlow and baseline methods on the OAS dataset. The **best** and <u>second best</u> results are marked. * means not desirable results with high diversity without matching the reference distribution. †: benchmarked results are quoted from [Mahajan et al., 2023] and [Frey et al., 2023].

Model	$W_{\text{property}}\downarrow$	Uniqueness ↑	$E_{ ext{dist}}(\hat{z},x)$	↑IntDiv ↑
dWJS †	0.065	0.97	62.7	65.1
L-WJS†	0.053	1.0	56.6	54.1
DEEN †	0.087	0.99	50.9	42.7
GPT3.5 †	0.140	0.66	55.4	46.1
IgLM †	0.087	1.0	48.6	34.6
ESM2†	0.150	1.0	70.99*	77.56*
ProtFlow	0.045	1.0	<u>58.6</u>	58.98

6 Conclusions

In this work, we introduced ProtFlow, the first flow matchingbased model for de novo protein sequence generation. Our approach leverages the straight and optimal probability path of rectified flow to achieve fast and high-quality sequence generation. By operating on a compressed and smoothed latent space derived from protein language models (pLMs), we enhanced the efficiency and accuracy of our method. Furthermore, the Reflow technique enabled high-quality one-step generation, significantly reducing computational costs. Additionally, we developed a multichain joint design pipeline and applied ProtFlow to various protein design tasks. ProtFlow consistently achieves state-of-the-art performance across all evaluated tasks with minimal generation steps, demonstrating its strong capability in rapidly designing high-quality protein sequences. For future work, exploration of scaling data, conditional generation strategies and multi-modality such as protein structure data can be introduced to extend the potential applications of ProtFlow to a much broader range.

References

- [Alamdari *et al.*, 2023] Sarah Alamdari, Nitya Thakkar, Rianne van den Berg, Alex Xijie Lu, Nicolo Fusi, Ava Pardis Amini, and Kevin K Yang. Protein generation with evolutionary diffusion: sequence is all you need. *bioRxiv*, pages 2023–09, 2023.
- [Bao et al., 2023] Fan Bao, Shen Nie, Kaiwen Xue, Yue Cao, Chongxuan Li, Hang Su, and Jun Zhu. All are worth words: A vit backbone for diffusion models. In Proceedings of the IEEE/CVF conference on computer vision and pattern recognition, pages 22669–22679, 2023.
- [Brown, 2020] Tom B Brown. Language models are few-shot learners. *arXiv preprint ArXiv:2005.14165*, 2020.
- [Chen *et al.*, 2024] Tianlai Chen, Pranay Vure, Rishab Pulugurta, and Pranam Chatterjee. Amp-diffusion: Integrating latent diffusion with protein language models for antimicrobial peptide generation. *bioRxiv*, pages 2024–03, 2024.
- [Consortium, 2019] UniProt Consortium. Uniprot: a worldwide hub of protein knowledge. *Nucleic acids research*, 47(D1):D506–D515, 2019.
- [Dao et al., 2023] Quan Dao, Hao Phung, Binh Nguyen, and Anh Tran. Flow matching in latent space. arXiv preprint arXiv:2307.08698, 2023.
- [Das et al., 2018] Payel Das, Kahini Wadhawan, Oscar Chang, Tom Sercu, Cicero dos Santos, Matthew Riemer, Vijil Chenthamarakshan, Inkit Padhi, and Aleksandra Mojsilovic. Pepcvae: Semi-supervised targeted design of antimicrobial peptide molecules. arXiv preprint arXiv:1810.07743, 2018.
- [Dhariwal and Nichol, 2021] Prafulla Dhariwal and Alexander Nichol. Diffusion models beat gans on image synthesis. *Advances in neural information processing systems*, 34:8780–8794, 2021.
- [Eijkelboom *et al.*, 2024] Floor Eijkelboom, Grigory Bartosh, Christian Andersson Naesseth, Max Welling, and Jan-Willem van de Meent. Variational flow matching for graph generation. *arXiv* preprint arXiv:2406.04843, 2024.
- [Frey et al., 2023] Nathan C Frey, Daniel Berenberg, Karina Zadorozhny, Joseph Kleinhenz, Julien Lafrance-Vanasse, Isidro Hotzel, Yan Wu, Stephen Ra, Richard Bonneau, Kyunghyun Cho, et al. Protein discovery with discrete walk-jump sampling. arXiv preprint arXiv:2306.12360, 2023.
- [Ho et al., 2020] Jonathan Ho, Ajay Jain, and Pieter Abbeel. Denoising diffusion probabilistic models. Advances in neural information processing systems, 33:6840–6851, 2020.
- [Hoogeboom et al., 2022] Emiel Hoogeboom, Victor Garcia Satorras, Clément Vignac, and Max Welling. Equivariant diffusion for molecule generation in 3d. In *International conference on machine learning*, pages 8867–8887. PMLR, 2022.

- [Hu et al., 2024] Vincent Hu, Di Wu, Yuki Asano, Pascal Mettes, Basura Fernando, Björn Ommer, and Cees Snoek. Flow matching for conditional text generation in a few sampling steps. In Proceedings of the 18th Conference of the European Chapter of the Association for Computational Linguistics (Volume 2: Short Papers), pages 380–392, 2024.
- [Huang *et al.*, 2016] Po-Ssu Huang, Scott E Boyken, and David Baker. The coming of age of de novo protein design. *Nature*, 537(7620):320–327, 2016.
- [Jhong *et al.*, 2019] Jhih-Hua Jhong, Yu-Hsiang Chi, Wen-Chi Li, Tsai-Hsuan Lin, Kai-Yao Huang, and Tzong-Yi Lee. dbamp: an integrated resource for exploring antimicrobial peptides with functional activities and physicochemical properties on transcriptome and proteome data. *Nucleic acids research*, 47(D1):D285–D297, 2019.
- [Kang et al., 2019] Xinyue Kang, Fanyi Dong, Cheng Shi, Shicai Liu, Jian Sun, Jiaxin Chen, Haiqi Li, Hanmei Xu, Xingzhen Lao, and Heng Zheng. Dramp 2.0, an updated data repository of antimicrobial peptides. *Scientific data*, 6(1):148, 2019.
- [Li et al., 2022] Xiang Li, John Thickstun, Ishaan Gulrajani, Percy S Liang, and Tatsunori B Hashimoto. Diffusion-lm improves controllable text generation. Advances in Neural Information Processing Systems, 35:4328–4343, 2022.
- [Lin et al., 2022] Zeming Lin, Halil Akin, Roshan Rao, Brian Hie, Zhongkai Zhu, Wenting Lu, Allan dos Santos Costa, Maryam Fazel-Zarandi, Tom Sercu, Sal Candido, et al. Language models of protein sequences at the scale of evolution enable accurate structure prediction. *BioRxiv*, 2022:500902, 2022.
- [Lin et al., 2023] Zeming Lin, Halil Akin, Roshan Rao, Brian Hie, Zhongkai Zhu, Wenting Lu, Nikita Smetanin, Robert Verkuil, Ori Kabeli, Yaniv Shmueli, et al. Evolutionary-scale prediction of atomic-level protein structure with a language model. *Science*, 379(6637):1123–1130, 2023.
- [Lipman *et al.*, 2022] Yaron Lipman, Ricky TQ Chen, Heli Ben-Hamu, Maximilian Nickel, and Matt Le. Flow matching for generative modeling. *arXiv preprint arXiv:2210.02747*, 2022.
- [Liu et al., 2022] Xingchao Liu, Chengyue Gong, and Qiang Liu. Flow straight and fast: Learning to generate and transfer data with rectified flow. arXiv preprint arXiv:2209.03003, 2022.
- [Lu et al., 2022] Cheng Lu, Yuhao Zhou, Fan Bao, Jianfei Chen, Chongxuan Li, and Jun Zhu. Dpm-solver: A fast ode solver for diffusion probabilistic model sampling in around 10 steps. Advances in Neural Information Processing Systems, 35:5775–5787, 2022.
- [Lu et al., 2024] Amy X Lu, Wilson Yan, Kevin K Yang, Vladimir Gligorijevic, Kyunghyun Cho, Pieter Abbeel, Richard Bonneau, and Nathan Frey. Tokenized and continuous embedding compressions of protein sequence and structure. bioRxiv, pages 2024–08, 2024.

- [Mahajan *et al.*, 2023] Sai Pooja Mahajan, Nathan C Frey, Daniel Berenberg, Joseph Kleinhenz, Richard Bonneau, Vladimir Gligorijevic, Andrew Watkins, and Saeed Saremi. Exploiting language models for protein discovery with latent walk-jump sampling. *Unknown*, 2023.
- [Meshchaninov et al., 2024] Viacheslav Meshchaninov, Pavel Strashnov, Andrey Shevtsov, Fedor Nikolaev, Nikita Ivanisenko, Olga Kardymon, and Dmitry Vetrov. Diffusion on language model embeddings for protein sequence generation. arXiv preprint arXiv:2403.03726, 2024.
- [Olsen *et al.*, 2022] Tobias H Olsen, Fergus Boyles, and Charlotte M Deane. Observed antibody space: A diverse database of cleaned, annotated, and translated unpaired and paired antibody sequences. *Protein Science*, 31(1):141–146, 2022.
- [Repecka *et al.*, 2021] Donatas Repecka, Vykintas Jauniskis, et al. Expanding functional protein sequence spaces using generative adversarial networks. *Nature Machine Intelligence*, 3(4):324–333, 2021.
- [Rombach *et al.*, 2022] Robin Rombach, Andreas Blattmann, Dominik Lorenz, Patrick Esser, and Björn Ommer. High-resolution image synthesis with latent diffusion models. *Unknown*, pages 10684–10695, 2022.
- [Ruffolo and Madani, 2024] Jeffrey A Ruffolo and Ali Madani. Designing proteins with language models. *nature biotechnology*, 42(2):200–202, 2024.
- [Saremi *et al.*, 2018] Saeed Saremi, Arash Mehrjou, Bernhard Schölkopf, and Aapo Hyvärinen. Deep energy estimator networks. *arXiv preprint arXiv:1805.08306*, 2018.
- [Shuai *et al.*, 2021] Richard W Shuai, Jeffrey A Ruffolo, and Jeffrey J Gray. Generative language modeling for antibody design. *BioRxiv*, pages 2021–12, 2021.
- [Song *et al.*, 2020a] Jiaming Song, Chenlin Meng, and Stefano Ermon. Denoising diffusion implicit models. *arXiv* preprint arXiv:2010.02502, 2020.
- [Song et al., 2020b] Yang Song, Jascha Sohl-Dickstein, Diederik P Kingma, Abhishek Kumar, Stefano Ermon, and Ben Poole. Score-based generative modeling through stochastic differential equations. arXiv preprint arXiv:2011.13456, 2020.
- [Stark et al., 2024] Hannes Stark, Bowen Jing, Chenyu Wang, Gabriele Corso, Bonnie Berger, Regina Barzilay, and Tommi Jaakkola. Dirichlet flow matching with applications to dna sequence design. arXiv preprint arXiv:2402.05841, 2024.
- [Steinegger and Söding, 2017] Martin Steinegger and Johannes Söding. Mmseqs2 enables sensitive protein sequence searching for the analysis of massive data sets. *Nature biotechnology*, 35(11):1026–1028, 2017.
- [Suzek *et al.*, 2015] Baris E Suzek, Yuqi Wang, Hongzhan Huang, Peter B McGarvey, Cathy H Wu, and UniProt Consortium. Uniref clusters: a comprehensive and scalable alternative for improving sequence similarity searches. *Bioinformatics*, 31(6):926–932, 2015.

- [Szymczak et al., 2023] Paulina Szymczak, Marcin Możejko, Tomasz Grzegorzek, Radosław Jurczak, Marta Bauer, Damian Neubauer, Karol Sikora, Michał Michalski, Jacek Sroka, Piotr Setny, et al. Discovering highly potent antimicrobial peptides with deep generative model hydramp. nature communications, 14(1):1453, 2023
- [Tong et al., 2023] Alexander Tong, Nikolay Malkin, Guillaume Huguet, Yanlei Zhang, Jarrid Rector-Brooks, Kilian Fatras, Guy Wolf, and Yoshua Bengio. Conditional flow matching: Simulation-free dynamic optimal transport. arXiv preprint arXiv:2302.00482, 2(3), 2023.
- [Valeriani et al., 2024] Lucrezia Valeriani, Diego Doimo, Francesca Cuturello, Alessandro Laio, Alessio Ansuini, and Alberto Cazzaniga. The geometry of hidden representations of large transformer models. Advances in Neural Information Processing Systems, 36, 2024.
- [Van Oort et al., 2021] Colin M Van Oort, Jonathon B Ferrell, Jacob M Remington, Safwan Wshah, and Jianing Li. Ampgan v2: machine learning-guided design of antimicrobial peptides. *Journal of chemical information and modeling*, 61(5):2198–2207, 2021.
- [Veltri *et al.*, 2018] Daniel Veltri, Uday Kamath, and Amarda Shehu. Deep learning improves antimicrobial peptide recognition. *Bioinformatics*, 34(16):2740–2747, 2018
- [Wang et al., 2024] Xinyou Wang, Zaixiang Zheng, Fei Ye, Dongyu Xue, Shujian Huang, and Quanquan Gu. Diffusion language models are versatile protein learners. arXiv preprint arXiv:2402.18567, 2024.
- [Wu *et al.*, 2022] Ruidong Wu, Fan Ding, Rui Wang, Rui Shen, Xiwen Zhang, Shitong Luo, Chenpeng Su, Zuofan Wu, Qi Xie, Bonnie Berger, et al. High-resolution de novo structure prediction from primary sequence. *BioRxiv*, pages 2022–07, 2022.
- [Yeh *et al.*, 2023] Andy Hsien-Wei Yeh, Christoffer Norn, Yakov Kipnis, Doug Tischer, Samuel J Pellock, Declan Evans, Pengchen Ma, Gyu Rie Lee, Jason Z Zhang, Ivan Anishchenko, et al. De novo design of luciferases using deep learning. *Nature*, 614(7949):774–780, 2023.
- [Yim *et al.*, 2024] Jason Yim, Andrew Campbell, Emile Mathieu, Andrew YK Foong, Michael Gastegger, José Jiménez-Luna, Sarah Lewis, Victor Garcia Satorras, Bastiaan S Veeling, Frank Noé, et al. Improved motif-scaffolding with se (3) flow matching. *ArXiv*, 2024.
- [Zhu et al., 2024a] Yiheng Zhu, Zitai Kong, Jialu Wu, Weize Liu, Yuqiang Han, Mingze Yin, Hongxia Xu, Chang-Yu Hsieh, and Tingjun Hou. Generative ai for controllable protein sequence design: A survey. arXiv preprint arXiv:2402.10516, 2024.
- [Zhu et al., 2024b] Yiheng Zhu, Jialu Wu, Qiuyi Li, Jiahuan Yan, Mingze Yin, Wei Wu, Mingyang Li, Jieping Ye, Zheng Wang, and Jian Wu. Bridge-IF: Learning inverse protein folding with markov bridges. In *The Thirty-eighth Annual Conference on Neural Information Processing Systems*, 2024.

A Models & Settings

A.1 Model Architecture

For the compressor and decompressor, we modified based on the settings of Hourglass Transformer [Lu et al., 2024]. We utilize the hidden size of 480 corresponding to the embedding of ESM-2 35M, the depth of 4, 8 attention heads, 64 dimensional heads and no hidden dropout rate. The pooling layer uses mean pooling and the activation layer uses tanh function. The linear layers make projections between hidden size of 480 and the compressed hidden size of 480/c. The two additional sets of norm and denorm layers are dimensional z-score and min-max normalizations.

The backbone of the flow matching holder module is a 12layer BERT model with 16 attention heads. We use the GELU activation function, with an intermediate layer size of 3072 and an attention dropout rate of 0.1. Depending on the ESM-2 variant employed, the hidden size is set to 320 when using ESM-2 8M and 480 when using ESM-2 35M, with a hidden dropout rate of 0.1. To match the compressed hidden size of the compressor and decompressor, we add two additional linear layer to make projections between hidden size of 480 and the compressed hidden size of 480/c. The noised protein sequence embeddings are added with positional encodings before being fed into the flow matching holder module. Timesteps are projected to match the size of the protein sequence embeddings using a sinusoidal embedding block, and these are added to the input of each Transformer block after a linear projection. We maintain the advanced long skip connections from [Meshchaninov et al., 2024], which involve adding the linear projections of earlier block inputs to those of later blocks.

A.2 Experimental Settings

For fine-tuning the ESM-2 decoder, the model is trained for 1 epoch with 1000 steps, and validation is performed every 50 steps. The learning rate is set to 0.00005. We use a batch size of 512 and the AdamW optimizer, with beta values of (0.9, 0.98) and a weight decay of 0.001.

For training of the compressor and decompressor, all experiments converge within 30 epoches. We perform validation using reconstrction accuracy on the validation set and save checkpoints every 5000 iterations. A cosine learning rate scheduler with two cycle limit is employed, featuring a minimum learning rate of 8e-5 and 10000 linear warmup steps. The batch size is set to 16 for both training and validation. The AdamW optimizer is configured with beta values of (0.9, 0.999).

For training of the flow matching holder, all experiments converge within 1,000,000 iterations. We perform validation using FPD on the validation set and save checkpoints every 10,000 iterations. A cosine learning rate scheduler with a single cycle limit is employed, featuring a minimum learning rate of 0.0002 and 5000 linear warmup steps. The batch size is set to 64 for peptides and AMPs training and validation, and set to 16 for general proteins and antibodies. The AdamW optimizer is configured with beta values of (0.9, 0.98), the weight decay of 0.01, the epsilon of 0.000001, and the gradient clipping norm of 1. We trained on one NVIDIA GeForce

RTX 3090 GPU. Training models on the general peptide and AMP task for 1,000,000 training steps takes around 42 hours while the general protein and antibody task needs 120 hours.

For inference, we test the Euler ODE solver with $N \in \{1,5,10,25\}$ steps for reflow and the Dormand Prince 45 ODE solver with $N \in \{1,5,10,25,50,75,100\}$ steps for the other tasks. The optimal solver and sampling steps are different for different tasks. Normally the Dormand Prince 45 ODE solver with a sampling step of 25 can give relatively good results with robustness. For reflow task, the model can support 1-step generation.

B Algorithms

B.1 Training procedure

Algorithm 1 Training ProtFlow with 1-RF

- 1: **Input:** data distribution π_0 , noise distribution ϵ , initial neural network v_{θ} , encoder Emb, compressor C, training step T.
- 2: **Output:** trained neural network v_{θ} .

```
3: for \bar{1}, 2, ..., i in T do 4: x_0 \sim \pi_0, x_1 \sim \epsilon
```

5:
$$x_0 \leftarrow \operatorname{padding}(x_0)$$

6: $x_0 \leftarrow Emb(x_0)$

7:
$$x_0 \leftarrow C(x_0)$$

8:
$$t \sim \text{Uniform}(0,1)$$

9:
$$x_t \leftarrow tx_1 + (1-t)x_0$$

10:
$$u_t \leftarrow x_1 - x_0$$

11:
$$\mathcal{L}_{CFM}(\theta) \leftarrow ||v_{\theta}(x_t, t) - u_t||_2^2$$

12:
$$\theta \leftarrow \text{Update}(\nabla \mathcal{L}_{\text{CFM}}(\theta), \theta)$$

13: **end for**

14: Return v_{θ} .

B.2 Inference procedure

Algorithm 2 ProtFlow Sampling

- 1: **Input:** data distribution π_0 , noise distribution ϵ , neural network v_{θ} , decompressor DeC, decoder D, sampling step N.
- 2: Output: generated samples x_0 .

```
3: x_1 \sim \epsilon
```

4:
$$x_t \leftarrow x_1$$

5: **for**
$$1, 2, ..., i$$
 in N **do**

6:
$$t \leftarrow 1/i$$

7:
$$x_t \leftarrow \text{ODE_Solver}(v_\theta(x_t, t), x_t, N)$$

8: end for

9:
$$x_0 \leftarrow x_t$$

10:
$$x_0 \leftarrow DeC(x_0)$$

11:
$$mask \sim \text{Length_Sampler}(pi_0)$$

- 12: $x_0 \leftarrow D(x_0, mask)$
- 13: Return x_0 .

B.3 ReFlow Training procedure

Algorithm 3 Training ProtFlow with 1-RF

```
v_{1-BF}, encoder Emb, compressor C, training step T,
      sampling step M, sampling step N.
 2: Output: trained reflow neural network v_{\theta}.
 4: for 1, 2, ..., i in M do
 5:
         x_1 \sim \epsilon
 6:
         x_t \leftarrow x_1
         for 1, 2, \ldots, i in N do
 7:
 8:
             x_t \leftarrow \text{ODE\_Solver}(v_\theta(x_t, t), x_t, N)
 9:
10:
11:
          z_0 \sim x_0, z_1 \sim x_1
         \pi.append((z_0, z_1))
12:
13: end for
14: for 1, 2, \ldots, i in T do
15:
          z_0, z_1 \sim \pi
16:
         t \sim \text{Uniform}(0,1)
17:
         z_t \leftarrow tz_1 + (1-t)z_0
         u_t \leftarrow z_1 - z_0
18:
         \mathcal{L}_{\text{CFM}}(\theta) \leftarrow \|v_{\theta}(z_t, t) - u_t\|_2^2
\theta \leftarrow \text{Update}(\nabla \mathcal{L}_{\text{CFM}}(\theta), \theta)
19:
20:
21: end for
```

1: **Input:** noise distribution ϵ , trained 1-RF neural network

C Experiment Details

C.1 General Protein Design

Baselines

22: Return v_{θ} .

ProteinGAN [Repecka *et al.*, 2021] represents a pioneering application of generative models in *de novo* protein sequence design. It is a variant of the generative adversarial network (GAN), where both the discriminator and generator are based on ResNet-based convolutional neural networks (CNNs), further enhanced with a self-attention layer. While ProteinGAN was originally developed for Multiple Sequence Alignment (MSA) tasks, which focus on specific protein families, it has also demonstrated competitive performance in our general peptide design task.

EvoDiff-OADM [Alamdari *et al.*, 2023] is the first foundation diffusion model for protein design trained on evolutionary-scale protein sequence data. EvoDiff grounds in two famous discrete diffusion frameworks: D3PM and OADM, while the OADM-based model is reported to perform better in the original paper. EvoDiff-OADM operates in an order-agnostic autoregressive manner, gradually converting single amino acids to or from the mask token during the forward or reverse processes. The model is based on the ByteNet CNN architecture, and we utilize a configuration with 38 million parameters. Furthermore, we evaluate its performance after replacing the backbone with the Transformer-based ESM-2 35M architecture.

DiMA [Meshchaninov *et al.*, 2024] is one of the earliest diffusion model operating on continuous space by embedding discrete protein sequences with the protein language model ESM-2 8M and surpasses lots of traditional mainstream generative models. It is built on a 12-layer transformer model

and introduces multiple novel techniques to improve the performance, including long skip connections, self-conditioning and tanh noise schedule.

Dirichlet Flow Matching [Stark *et al.*, 2024] is a pioneering approach in discrete flow matching, framing generative modeling as a transportation problem on the probability simplex and using the Dirichlet distribution as the probability distribution for the generative path. It has demonstrated strong performance in DNA sequence design tasks. We adapted Dirichlet Flow Matching from the DNA sequence design into the amino acid sequence generation of protein, retaining the original CNN architecture and model hyperparameter settings.

Metrics

Predicted Local Distance Difference Test (pLDDT) is a metric used to assess the accuracy of protein structure predictions. It reflects the foldability and structural plausibility of a protein sequence by predicting the confidence score for each residue position in the protein structure. We utilize OmegaFold [Wu *et al.*, 2022] to predict the pLDDT of each residue of the sequence and average them as the pLDDT of that sequence.

ESM-2 pseudoperplexity (**ESM-2 pppl**) measures how well a given protein sequence aligns with the patterns the assessing model has learned from the training data of ESM-2, which is a large scale standard protein database UniRef50 [Suzek *et al.*, 2015]. We calculate ESM-2 pppl with ESM-2 35M [Lin *et al.*, 2023] by masking each amino acid of the protein sequence and predicting it considering all the other amino acids in the sequence, and calculating the value with the equation

ESM-2 pseudoperplexity =
$$\exp\left(-\frac{1}{|x|}\sum_{i=1}^{|x|}\log p(x_i\mid x_{j\neq i}, \theta_{\text{ESM-2}})\right)$$

where |x| means the length of sequence x and $x_{j\neq i}$ means sequence x without the i^th amino acids.

Self-consistency perplexity (scPerplexity) provides a measurement of sequence reliability and quality in a structure-based aspect. After predicting the structure of each sequence with OmegaFold [Wu *et al.*, 2022], we utilize ESM-IF 142M to inverse fold the structure, which predicts a sequence that would naturally fold into that structure. Then we compute the perplexity against the original generated sequence.

TM-Score evaluates the similarity between two protein pairs in structure. Compared to pLDDT which also make evaluations on the structure level, TM-Score focuses more on the global level. For each protein sequence, after predicting the structure with OmegaFold, we use the FoldSeek easy-search tool to seek for the most similar protein in the AlphaFold SwissProt database with the TM-Score function

$$\text{TM-score} = \frac{1}{|x_{query}|} \sum_{i=1}^{|x_{query}|} \frac{1}{1 + \left(\frac{d_i}{\sigma |x_{target}|}\right)^2}$$

where $|x_{query}|$ means the number of aligned residues between the query and target proteins, $|x_{target}|$ is the length of

the target protein, σ is a scaling factor, and d_i is the distance between the i^{th} aligned residue pairs.

Fréchet ProtT5 Distance (FPD) is a variant of Fréchet distance (FD), which measures the dissimilarity between two samples drawn from multivariate Gaussian distributions. Given two samples $X_1 \sim \mathcal{N}(\mu_1, \Sigma_1)$ and $X_2 \sim \mathcal{N}(\mu_2, \Sigma_2)$, the FID can be calculated as

Fréchet Distance =
$$\|\mu_1 - \mu_2\|^2 + \operatorname{tr}(\Sigma_1 + \Sigma_2 - 2\sqrt{\Sigma_1\Sigma_2})$$

We calculate the Fréchet distance of protein sequence embeddings using protein language model ProtT5, namely, Fréchet ProtT5 Distance (FPD).

Maximum mean discrepancy (MMD) is a kernel-based statistical test to determine whether two samples $X = \{x_1, x_2, \ldots, x_n\}$ and $Y = \{y_1, y_2, \ldots, y_n\}$ belong to different distributions. Suppose the kernel is k, then MMD can be calculated as

$$MMD = \frac{1}{n^2} \sum_{i=1}^{n} \sum_{j=1}^{n} (k(x_i, x_j) + k(y_i, y_j) - 2k(x_i, y_j))$$

We calculate MMD on the ProtT5 embeddings and use the radial basis function kernel.

1-Wasserstein optimal transport (OT) evaluates the similarity between two batches of sequences. We use pairwise Levenshtein distances as transportation costs, utilize the Earth Mover Distance (EMD) solver with a uniform distribution of the samples to determine optimal sequence pairs, and take the average of the distances between optimal pairs.

C.2 Antimicrobial Peptide Design

Baselines

HydrAMP [Szymczak *et al.*, 2023] is based on a conditional variational autoencoder (cVAE) with an autoencoder and a decoder, and incorporates a pre-trained classifier. It captures the antimicrobial properties of peptides by learning their low-dimensional, continuous representations and decouples these properties from the antimicrobial conditions. The model is designed to generate peptide sequences that meet specific antimicrobial activity criteria. HydrAMP can perform both unconstrained generation and generate antimicrobial analogs based on provided prototype peptides.

PepCVAE [Das *et al.*, 2018] is a semi-supervised generative model also based on a cVAE and incorporates a pretrained Antimicrobial Peptide (AMP) classifier. The model learns a rich latent space representation by leveraging a large dataset of unlabeled peptide sequences along with a smaller set of sequences labeled as either antimicrobial or non-antimicrobial. By decoupling the antimicrobial properties from the latent space, PepCVAE is able to generate peptide sequences that meet specific antimicrobial objectives.

AMPGAN [Van Oort et al., 2021] is based on a Bidirectional Conditional Generative Adversarial Network (BiC-GAN). In addition to the standard generator and discriminator components found in typical GANs, it introduces an encoder that maps real data into the generator's latent space. This enables iterative peptide sequence generation, optimization, interpolation, and incremental modifications. The generation process in AMPGAN is controlled by conditioning variables,

allowing the discriminator to learn the relationship between relevant antimicrobial features and the generated peptide sequences.

AMP-Diffusion [Chen et al., 2024] is built on the continuous diffusion model framework, where peptide sequences are mapped into a latent space using the protein language model ESM-2 for the diffusion process. The model architecture incorporates pre-trained ESM-2 8M attention blocks for the denoising process and a multilayer perceptron (MLP) as the output layer. The timestep is embedded with a positional encoding and integrated into protein embeddings with a scaling factor and a bias adjustment. AMP-Diffusion reports a leading performance compared to mainstream AMP design methods and is able to generate AMPs with good predicted activities.

Metrics

ESM-2 pseudoperplexity is utilized again to evaluate the sequence quality. The measurement settings keep the same with the ESM-2 pppl of the general peptide design task.

Shannon Entropy is a concept from information theory measuring the uncertainty of information source or randomness of information, reflecting the information content and complexity. In protein sequence design tasks, Shannon entropy can indicate the diversity of single protein sequence. For each peptide sequence, we compute the probability of occurrence of each amino acid $p(x_i)$, $i \in \{0, 1, 2, \dots, 19\}$, and calculate the Shannon entropy as

$$H(X) = -\sum_{i=1}^{n} p(x_i) \log_2 p(x_i)$$

Jaccard Similarity Coefficient is a widely used measurement to evaluate the similarity of two sets. We use 6-mers Jaccard similarity coefficient (JS-6) to compare the similarity of generated peptide sets to the training data. We split the training dataset and the generated peptide set with 6-mers, which represents all the sub-sequences with length 6 in the dataset to be processed. Then the Jaccard similarity can be calculated with the equation

$$\operatorname{Jaccard}(A, B) = \frac{|A \cap B|}{|A \cup B|}$$

where A represents the 6-mers set of training dataset and B is the 6-mers set of generated batch set.

External Classifier can be an additional validation tool to ascertain the antimicrobial properties of generated peptides. We use the classifier of HydrAMP [Szymczak *et al.*, 2023], which consists of two distinct networks predicting the probability of a peptide being antimicrobial, and the minimal inhibitory concentration (MIC) against *E. coli*, a significant kind of bacteria serving as a model organism. We utilize the HydrAMP classifiers to score each peptide generated, and calculate the proportion of generated peptides with AMP score greater than 0.8 as P_{amp} and MIC score greater than 0.5 as P_{mic} .

C.3 Antibody Design

Baselines

dWJS [Frey *et al.*, 2023] is a discrete generative model that combines Energy-Based Models (EBMs) with score-based

models. The dWJS method is built upon a novel Smoothed Discrete Sampling (SDS) framework. The model trains the EBM by applying maximum likelihood estimation on noisy data and introduces a Walk-Jump sampling-denoising mechanism. Specifically, it utilizes Langevin MCMC to sample from the smoothed noisy data distribution, followed by a denoising step using a separately trained neural network. dWJS is primarily applied in antibody design, where it efficiently generates high-quality and novel samples.

L-WJS [Mahajan *et al.*, 2023] introduces a single-step, score-based diffusion framework for antibody sequence design from higher dimensional embeddings of pretrained language models (pLMs). It also utilize the Walk-Jump sampling-denoising mechanism of dWJS but it sampling on the continuous space of pLMs.

representations of sequences. All modules are trained simultaneously.

DEEN [Saremi *et al.*, 2018] can be thought of as an energy parameterization of a score-based diffusion model. DEEN is based on MLPs that are used to estimate the energy of the data distribution. This energy function is learned by minimizing a scalable objective function that is constructed using score matching, thereby avoiding the direct computation of the partition function. Moreover, DEEN is not trained with contrastive divergence, so the EBM formulation is completely distinct in terms of parameterization, training, and sampling.

IgLM [Shuai *et al.*, 2021] is a GPT2-style transformer-based language model trained specifically for antibody design. It was trained on 558M antibody heavy chain and light chain sequences from the Observed Antibody Space (OAS) database. The input sequences to the model are tagged with conditional labels that indicate the type of chain (heavy or light) and the source species, allowing the generated sequences to be controlled based on specific species and chain types.

ESM-2 [Lin *et al.*, 2023] is a large-scale protein language model with 15B parameters. It is built on a Transformer architecture and employs relative position encoding to handle sequences of varying lengths, enhancing the model's ability to generalize across sequences of any length. The model is trained on protein sequence data using the Masked Language Model (MLM) task, where it predicts the identity of randomly masked amino acids. ESM-2 is capable of making highly accurate protein structure predictions based solely on the protein sequence. However, dWJS [Frey *et al.*, 2023] reports that since ESM-2 is not trained for antibody generation, it generates highly repetitive sequences that are very dissimilar to antibodies. Then its leading metric scores are therefore meaningless.

Additionally, the famous large language model on general tasks, GPT3.5, is also taken into consideration of the baseline.

Metrics

Properties Wasserstein distance ($W_{property}$) is the normalized average Wasserstein distance between the property distributions of generated samples and the validation set, reflecting the learning quality of the training data distribution. For each antibody sequence of the heavy chain, we first calculate fifteen biological propeties with the biopython, including the

sequence length, molecular weight, aromaticity, instability index, isoelectric point, gravy, charge at pH6, charge at pH7, helix fraction, turn structure fraction, sheet structure fraction, molar extinction coefficient reduced, molar extinction coefficient oxidized, average hydrophilicity, and average surface accessibility. For each property distribution of the generated sequences and validation datasets, we calculate the Wasserstein distance using scipy after minmax normalization. The final Wasserstein distance is the average of the Wasserstein distance of these 15 property distributions.

Uniqueness is a simple indicator evaluating the novelty of the generated sequences. It is the fraction of unique sequences in the generated sequences.

Edit distance (E_{dist}) , also known as the Levenshtein distance, is a metric used to measure the similarity between two strings. It represents the minimum number of edit operations required to transform one string into another. The edit distance of sequence s_1 and s_2 can be calculated use the recursive formula

$$d(i,j) = \begin{cases} 0 & \text{if } i = 0 \text{ and } j = 0 \\ j & \text{if } i = 0 \\ i & \text{if } j = 0 \\ d(i-1,j)+1 & \text{if } s_1[i] \neq s_2[j] \text{ and replace} \\ d(i,j-1)+1 & \text{if } s_1[i] \neq s_2[j] \text{ and insert} \\ d(i-1,j-1) & \text{if } s_1[i] = s_2[j] \end{cases}$$

where d(i, j) means the minimum number of edit operations required to transform the first i characters of s_1 into the first j characters of s_2 . We utilize the mean of the edit distance of sampled sequences from the validation set, which summarizes the novelty and diversity of samples compared to the validation set.

Internal diversity (IntDiv) is the mean of the edit distance within the generated sequences, reflecting the diversity within the distribution of generated samples. For each sequence, we calculate the edit distance from the rest of the generated sequences and take the average.

D Additional Visualization of Generated Proteins

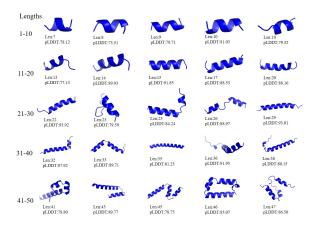


Figure 3: Visualized examples of general peptide design.

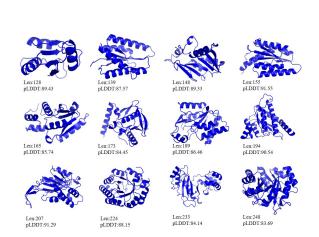


Figure 4: Visualized examples of general long-chain protein design.

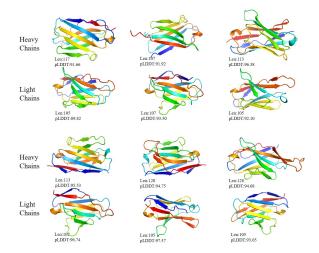


Figure 6: Visualized examples of antibody design.



Figure 5: Visualized examples of AMP design.