LatentDE: Latent-based Directed Evolution accelerated by Gradient Ascent for Protein Sequence Design

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Abstract

Directed evolution is a powerful but resource-intensive method for optimizing protein functionalities by screening a vast range of mutations. Recent advances in machine learning aim to streamline this process by using surrogate sequence-function models. We propose Latent-based Directed Evolution (LDE), an evolutionary algorithm that efficiently explores high-fitness mutants in the latent space. Built on a regularized variational autoencoder (VAE) and the state-of-the-art Protein Language Model ESM-2, LDE creates a meaningful latent representation of sequences and combines gradient-based methods with directed evolution for effective fitness landscape traversal. Our experimental results on eight protein design tasks show that LDE outperforms existing baseline algorithms. Our source code is publicly available at `https://github.com/HySonLab/LatentDE`.

1 Introduction

Protein engineering aims to design proteins with specific functions, determined by their amino acid sequences that fold into three-dimensional structures [15, 11], creating a fitness landscape that maps sequences to functions [54]. Inspired by natural evolution, *directed evolution* [2] iteratively mutates and selects protein variants to optimize desired functions.

Recent machine learning (ML) methods have enhanced the efficiency of evolutionary searches for protein design [33, 52, 34, 13]. However, these methods often rely on costly, repetitive rounds of mutagenesis and validation in a vast, discrete sequence space, making them prone to local optima and false positives [6, 12, 5]. Latent space optimization (LSO) offers a more efficient alternative by using continuous, low-dimensional representations, but its integration with directed evolution remains underexplored.

Protein design is a black-box model-based optimization (MBO) problem, where the objective is to identify sequences maximizing fitness. While online MBO iteratively proposes designs based on feedback, its effectiveness is limited by the availability of experimental data. Offline MBO, which relies on a static dataset, is more efficient but can struggle with out-of-distribution inputs, causing the learned model $\hat{f}(x)$ to predict fitness inaccurately [27].

To address these challenges, we propose **L**atent-based **D**irected **E**volution (LDE), the first method to integrate directed evolution within a latent space. Leveraging the latent space of ESM-2 [28], LDE uses a variational autoencoder (VAE) to reconstruct and predict the fitness of sequences. Combining the strengths of both MBO approaches, LDE first uses gradient ascent to guide latent representations toward high-fitness regions and then integrates directed evolution by introducing noise to explore

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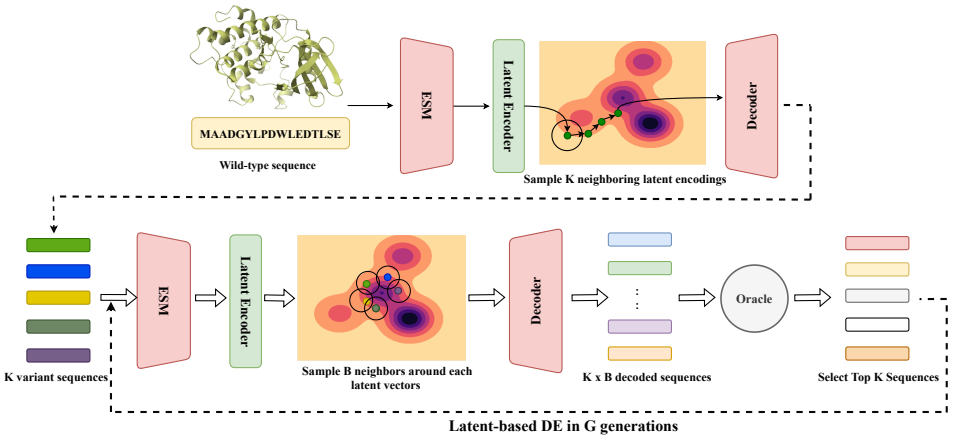


Figure 1: Overview of our proposed method. **Top**: Encoding of the wild-type sequence into latent variables and gradient ascent towards high-fitness regions. **Bottom**: Latent-based directed evolution performed over G generations, sampling B neighbors around the latent variables for evaluation by a black-box oracle.

locally. This process enables efficient sampling and optimization of sequences using oracles such as wet-lab experiments or ML models.

2 Method

Optimizing the latent variables of generative models has proven effective in various drug and protein design tasks [20, 42]. Our work introduces a novel approach that utilizes directed evolution to efficiently discover optimal protein sequences directly from their latent representations. The theoretical background for Directed Evolution (DE) and Latent Space Optimization (LSO), which form the basis of our approach, is discussed in detail in Appendix A. We begin with the problem formulation in Section 2.1. Then, we present our pre-trained regularized variational autoencoders (VAEs) [23] in Section 2.2 and how to perform directed evolution, which is accelerated by gradient ascent, in the latent space of VAEs in Section 2.3. We describe our model architecture in Appendix B.

2.1 Problem Formulation

We aim to find high-fitness protein sequences s in the sequence space $\mathcal{V}^L$, where $\mathcal{V}$ is the vocabulary of amino acids and L is the sequence length. The goal is to maximize a black-box protein fitness function $\mathcal{O} : \mathcal{V}^L \mapsto \mathbb{R}$, which can only be evaluated through wet-lab experiments:

$$x^* = \text{argmax}_{x \in \mathcal{V}^L} \mathcal{O}(x). \tag{1}$$

For in-silico evaluation, we use an oracle $\mathcal{O}_\psi$ parameterized by ψ to approximate $\mathcal{O}$. Given a training subset $\mathcal{D}_t$, our task is to generate sequences $\hat{x}$ that optimize the fitness predicted by $\mathcal{O}_\psi$. We focus on designing sequences starting from a wild-type sequence and iteratively generating candidates with improved properties, as illustrated in Figure 1.

Algorithm 1 Latent-based directed evolution accelerated by gradient ascent

Input: x^{wt}, encoder ϕ, decoder θ, fitness predictor f, # iterations T, # generations G, beam size B, oracle $\mathcal{O}$.

$\mathcal{P}_0 \leftarrow \emptyset$
for $i = 1$ **to** K **do**
  Sample $z \sim \mathcal{N}(\mu_\phi(x^{wt}), \sigma_\phi(x^{wt})^2)$
  $z_T \leftarrow$ gradient_ascent(z, f, T) in Equation (5)
  $\hat{x} \leftarrow \theta(z_T)$
  $P_0 \leftarrow P_0 \cup \{(\hat{x}, \mathcal{O}(\hat{x}))\}$
end for
for $g = 1$ **to** G **do**
  $P_g \leftarrow \emptyset$
  for $k = 1$ **to** K **do**
    for $b = 1$ **to** B **do**
      Sample $z_{k,b} \sim \mathcal{N}(\mu_\phi(x_k), \sigma_\phi(x_k)^2)$
      $p \sim \mathcal{U}[0, 1)$
      if $p >$ threshold **then**
        Inject noise to $z_{k,b}$ based on Equation (6)
      end if
      $\hat{x}_{k,b} \leftarrow \theta(z_{k,b})$
      $P_g \leftarrow P_g \cup \{(\hat{x}_{k,b}, \mathcal{O}(\hat{x}_{k,b}))\}$
    end for
    $P_g \leftarrow P_g \cup P_{g-1}$
    $P_g \leftarrow$ topK(P_g)
  end for
end for

2.2 Regularized Variational Autoencoder

Given a dataset $\mathcal{D}_t = \{(x_i, y_i)\}_{i=1}^N$ of protein sequences $x_i \in \mathcal{V}^L$ with fitness values $y_i \in \mathbb{R}$, we train a VAE with an encoder $\phi : \mathcal{V}^L \mapsto \mathbb{R}^{d_h}$ and a decoder $\theta : \mathbb{R}^d \mapsto \mathcal{V}^L$. The VAE encodes each sequence into a latent vector $z_i \in \mathbb{R}^d$ sampled from $\mathcal{N}(\mu_i, \sigma_i^2)$ and reconstructs it back to $\hat{x}_i \in \mathcal{V}^L$. The training objective combines cross-entropy loss $\mathcal{C}(\hat{x}_i, x_i)$ and Kullback-Leibler (KL) divergence:

$$\mathcal{L}_{vae} = \frac{1}{N}\sum_{i=1}^{N} \mathcal{C}(\hat{x}_i, x_i) + \frac{\beta}{N}\sum_{i=1}^{N} D_{\text{KL}}(\mathcal{N}(\mu_i, \sigma_i^2) \| \mathcal{N}(0, I_d)). \tag{2}$$

where β controls the disentanglement of the VAE's latent space.

Latent Space Regularization The KL-divergence regularizer in Equation (2) encourages the encoder ϕ to produce a latent space close to the unit Gaussian prior. However, this may reduce the meaningfulness of latent representations [9]. To address this, following prior works [8, 16], we jointly train the VAE with a fitness predictor $f : \mathbb{R}^d \mapsto \mathbb{R}$ to enhance the separation of high-fitness representations:

$$\mathcal{L} = \mathcal{L}_{vae} + \frac{1}{N}\sum_{i=1}^{N} ||f(z_i) - y_i||_2^2, \tag{3}$$

where y_i is the experimental fitness of sequence x_i, and $z_i = \phi(x_i)$. This L_2 regularization aligns latent representations with their fitness scores.

2.3 Latent-Based Directed Evolution

Inspired by nature's evolutionary process, where subtle mutations in a protein's sequence enhance fitness, our method explores the latent space around the wild-type sequence through iterative sampling from the pretrained encoder ϕ.

A key challenge is that the latent representation z of x^{wt} may reside in low-fitness regions, slowing convergence. To address this, we use gradient ascent to initialize the population $\mathcal{P}_0$, guiding the search toward higher fitness areas. Algorithm 1 outlines our approach, with further details on the evolutionary process and sampling techniques provided in Appendix C.

2.4 Efficient Sampling and Optimization

Traditional directed evolution is effective but computationally intensive for protein design. Our latent-based algorithm improves sampling efficiency by compressing long sequences into low-dimensional latent representations via VAEs, simplifying the mutation process as noise addition in latent space. This approach, unlike complex mutation operators [3, 46], requires no domain knowledge and is more computationally efficient. The gradient ascent benefits from VAE regularization, creating a smoother optimization landscape and reducing the risk of local optima. Furthermore, even if the optimization converges to a local optimum, latent-based directed evolution (DE) can explore surrounding regions through controlled random perturbations for a broader search, enabling a more comprehensive search of promising areas.

3 Experiments

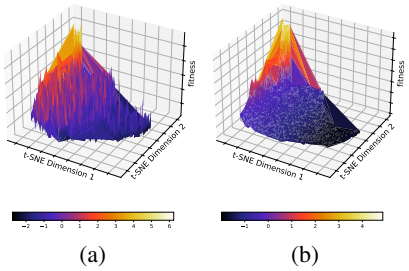
We conducted a comprehensive set of experiments to evaluate the effectiveness of our proposed LDE in protein sequence design. The experimental setup, including datasets, implementation details, baseline algorithms, oracles, and evaluation metrics, is described in Appendix D. Complete results of these experiments are provided in Appendix E.

Comparison with Baseline Algorithms As shown in Table 1, LDE outperforms other algorithms across eight protein benchmarks, especially for longer sequences (e.g., avGFP with 237 amino acids). This highlights LDE's advantage in efficiently exploring complex, high-dimensional protein spaces. While AAV contains shorter sequences (up to 28 amino acids), LDE's latent representations still enhance optimization. The diversity and novelty metrics in Tables 3 and 4 provide further insights into the exploration-exploitation trade-offs of various methods. It is important to note that maximizing these metrics does not always indicate better overall performance, as different algorithms have different objectives (e.g., optimizing fitness scores, maximizing diversity) [41, 35, 24]. For example, GFN-AL and COMs achieve high diversity but low fitness scores in the AMIE dataset.

Table 1: Maximum fitness scores across eight protein datasets. Shaded rows indicate the result of ablation studies.

	avGFP	AAV	TEM	E4B	AMIE	LGK	Pab1	UBE2I
x^{wt}	1.408	-6.778	-0.015	0.774	-2.789	-1.260	0.014	-0.262
AdaLead [40]	3.323	-1.545	0.248	-0.373	-1.483	-0.047	0.382	2.765
DyNA PPO [1]	5.331	-2.817	0.570	-0.575	-2.790	-0.060	0.183	2.630
CbAS [5]	5.187	-2.800	0.481	-0.658	-1.784	-0.056	0.276	2.693
CMA-ES [17]	5.125	-3.267	0.590	-0.658	-2.790	-0.086	0.254	2.527
COMs [45]	3.544	-3.533	0.472	-0.860	-20.182	-0.087	0.156	2.086
PEX [35]	3.796	2.378	0.252	4.317	-0.364	0.009	1.326	3.578
GFN-AL [19]	5.028	-4.444	0.654	-0.831	-37.360	-5.738	1.399	3.850
GGS [24]	3.368	2.442	1.121	-1.147	-3.364	-0.972	0.059	4.101
LDE (ours)	**8.058**	**2.636**	**1.745**	**5.120**	-0.103	**0.018**	1.548	**4.297**
– w/o GA	6.407	2.148	1.220	4.597	**-0.099**	-0.531	**1.592**	3.254
– w/o DE	3.677	0.919	-0.024	3.052	-0.701	-1.597	0.285	0.766

Impact of Gradient Ascent and Directed Evolution An ablation study demonstrates the contribution of each component of LDE. Variants without gradient ascent (w/o GA) or without directed evolution (w/o DE) show reduced performance, as seen in the two bottom lines of Table 1. This confirms the importance of combining gradient ascent for initialization with latent-based DE to achieve optimal results.



(a) (b)

Figure 2: The fitness landscape approximated by regularized VAEs of E4B proteins.

Latent Space Visualization Figures 2a and 2b illustrate the approximated fitness landscapes of protein sequences in the E4B family. These suggest that applying VAE regularization, as in Equation (3), organizes latent representations by fitness. Figure 2a demonstrates that our VAEs generate meaningful latent representations based on true fitness values, while Figure 2b effectively approximates a smooth fitness landscape, enabling effective gradient-based optimization methods. These results highlight the effectiveness of our latent-based directed evolution approach.

4 Conclusion and Future Work

In this work, we present Latent-based Directed Evolution (LDE), a novel method that combines directed evolution with gradient ascent in a regularized VAE latent space to efficiently optimize and design protein sequences. This approach leverages deep representation learning capabilities of generative models to significantly speed up the evolutionary process, achieving superior results compared to traditional methods solely operating in sequence space. LDE holds significant promise for accelerating protein engineering and drug discovery efforts, and we invite further research on integrating it with in vitro protein characterization for real-world validation.

On-going Direction However, our present work is not complete and has limitations, including its reliance on a single fitness predictor that could destabilize the optimization if poorly calibrated. To address this, we are planning to use ensembles of surrogate models with risk-aware strategies to handle uncertainty, along with robustness checks and sensitivity analyses to examine model stability and parameter impact. Previous studies [32, 49] have explored this issue and shown promising results. A potential extension would involve integrating structural information [47, 44] into the optimization process, ensuring that the generated structures closely resemble the wild-type to maintain functionality. Additionally, LDE could be adapted for multi-objective optimization. These ongoing efforts will enhance the robustness, applicability, and effectiveness of our method, connecting computational predictions with real-world biological outcomes.

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A Preliminaries

A.1 Directed Evolution Theory

Directed evolution (DE) is a widely used approach in protein engineering, focusing on identifying optimal protein sequences from a large set of unlabeled candidates $\mathcal{S}$ with minimal experimental

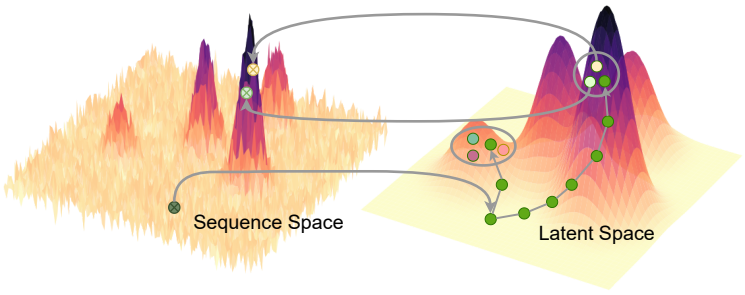

Figure 3: We perform directed evolution within the smooth and continuous latent space of a generative model. Our algorithm begins by identifying high-fitness regions through gradient ascent. Within these regions, we strategically sample a defined number of neighboring points (gray circles) to create a diverse population for the evolutionary process. This population then undergoes iterative selection and mutation within the latent space, ultimately converging to sequences with enhanced fitness.

validation [21, 48]. DE involves iterative cycles of mutation and selection, where protein variants with enhanced properties are chosen for subsequent rounds. This process effectively explores local regions of high-fitness proteins within the vast sequence space, capitalizing on the clustering of functional proteins observed in [30]. As depicted in the left part of Figure 3, DE begins with wild-type sequences, applying targeted mutations to discover variants with improved functions.

A.2 Latent Space Optimization

Latent space optimization (LSO) is a model-based technique that operates within the latent space $\mathcal{Z}$ of deep generative models. Here, an objective function $f : \mathcal{Z} \mapsto \mathbb{R}$ predicts objective values directly from latent representations. LSO is particularly effective when $\mathcal{Z}$ is low-dimensional and continuous, simplifying optimization challenges typically encountered in discrete, high-dimensional spaces. This transformation enables the application of techniques like gradient ascent and Bayesian optimization (BO) [38]. Moreover, f is trained using an encoder $\phi : \mathcal{X} \mapsto \mathcal{Z}$, which maps an input sample $x \in \mathcal{X}$ to its corresponding latent representation $z \in \mathcal{Z}$.

B Implementation Details

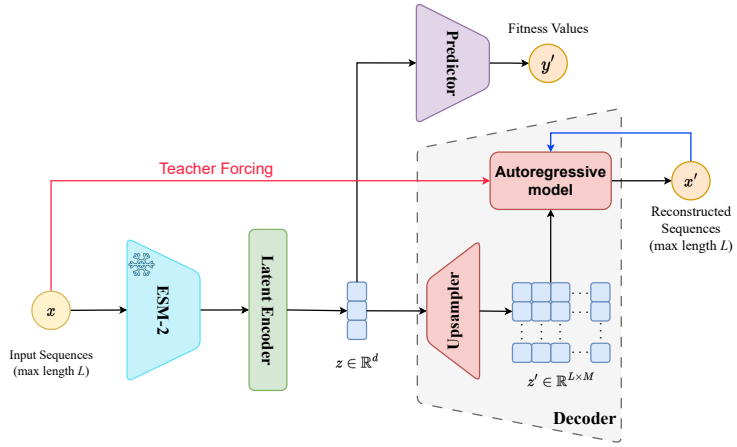

Figure 4: Schematic illustration of the VAE model used in the study.

B.1 VAEs' Architecture

In this section, we go into detail regarding the architecture of the VAE used in our study. As mentioned in the main text, our regularized VAE consists of an encoder, a predictor, and a decoder.

Encoder Figure 4 depicts that the encoder incorporates a pre-trained ESM-2 [28] followed by a latent encoder to compute the latent representation z. In our study, we leverage the powerful representation of the pre-trained 30-layer ESM-2 by making it the encoder of our model. Given an input sequence $x = \langle x_0, x_1, \cdots, x_L \rangle$, where $x_i \in \mathcal{V}$, the transformer-based ESM-2 computes representations for each token x_i in x, resulting in a token-level hidden representation $H = \langle h_0, h_1, \cdots, h_L \rangle, h_i \in \mathbb{R}^{d_h}$. We calculate the global representation $h \in \mathbb{R}^{d_h}$ of x via a weighted sum of its tokens:

$$h = \sum_{i=1}^{L} \frac{\omega^T \exp(h_i)}{\sum_{i=1}^{L} \omega^T \exp(h_i)} h_i. \tag{4}$$

here, ω is a learnable global attention vector. Then, two multi-layer perceptrons (MLPs) are used to compute $\mu = \text{MLP}_1(h)$ and $\log \sigma = \text{MLP}_2(h)$, where the latent dimension is d. Finally, a latent representation $z \in \mathbb{R}^d$ is sampled from $\mathcal{N}(\mu, \sigma^2)$, which is further proceeded to the decoder to reconstruct the sequence $\hat{x}$. We use an auxiliary MLP as a **fitness predictor** that maps the latent z to the fitness score, i.e. $y' = \text{MLP}(z)$. The hidden dimensional size of the predictor is set to 512, with the dropout of 0.2. We set $d_h = 1280$ and $d = 320$ for the main experiments in our study.

Decoder Inspired from [37], we construct our decoder as the combination of two components: an 'upsampler' component comprising 3 layers of transposed convolutions with stride of 2 to upsample the latent vector z to a sequence matching the output sequence's length; and an autoregressive component consisting of an attention-based GRU [10, 29] with 512 units. To cope with the optimization difficulties reported when training VAE with powerful autoregressive decoders [4, 37], we follow [4] by applying 40% dropout to the amino acid context supplied as input to the GRU during training. This encourages the network to depend on the information conveyed through the upsampled latent code along with the conditional information in the masked amino acid sequence to make predictions. Additionally, we apply teacher forcing [51] with a ratio of 50% for faster convergence.

B.2 Oracles

We establish the optimization oracle $\mathcal{O}(\cdot)$, utilized for latent-based directed evolution, by leveraging features generated by the pre-trained 33-layer ESM-2 with a dimension of 1280. Subsequently, we fine-tune an Attention1D model to predict fitness values based on these representations. As for the evaluation oracle $\mathcal{E}(\cdot)$, which acts as the "ground-truth" evaluator, we employ the trained oracle provided by [35] to assess all methods.

B.3 Training Configurations

We employ the ControlVAE mechanism to prevent KL vanishing and enhance the diversity of generated data during training. Across all tasks, we configure the coefficients K_p and K_i of the P term and I term to 0.01 and 0.0001, respectively. For the LGK benchmark, the desired KL-divergence C is set to 40, while for all other tasks, $C = 20$ is utilized. The batch size for each task is determined to be as large as possible, as long as the total steps in one epoch for each task are higher than 100.

C Detailed Methodology

Gradient Ascent (GA) At the beginning of our algorithm, the wild-type protein sequence x^{wt} is encoded by the encoder ϕ to produce its mean $\mu_\phi(x^{wt})$ and log variance $\log \sigma_\phi(x^{wt})$. A latent representation z is then sampled from $\mathcal{N}(\mu_\phi(x^{wt}), \sigma_\phi(x^{wt})^2)$. Subsequently, we perform gradient ascent with a learning rate of α in T iterations to move z to high-fitness regions as:

$$z_{t+1} = z_t + \alpha \nabla_z f(z)|_{z=z_t}, 0 \leq t < T, \tag{5}$$

where $\nabla_z f(z)|_{z=z_t}$ is the gradient of the fitness predictor f with respect to z_t. After T iterations, we add z_T to the initial population $\mathcal{P}_0$. The procedure is iteratively executed until K pairs of decoded sequences along with their respective fitness scores are obtained. At this stage, the fitness scores are computed by the latent predictor f, allowing fast computation and efficient latent sampling (see lines 1 to 7).

Evolutionary Process From lines 8 to 23, a latent-based directed evolution is conducted in G generations to generate K protein sequences with high fitness values. For each candidate x_k,

we sample B latent variables from its posterior distribution, where each is denoted as $z_{k,b} \sim \mathcal{N}(\mu_\phi(x_k), \sigma_\phi(x_k)^2)$. These latent representations are then decoded into sequences by the decoder θ, i.e. $\hat{x}_{k,b} = \theta(z_{k,b})$. Wet-lab experiments then evaluate the decoded sequences to obtain their fitness scores. All sequences generated in generation g are added into $\mathcal{P}_g$. Finally, at the end of g, the top K sequences are selected from both $\mathcal{P}_{g-1}$ and $\mathcal{P}_g$.

Random Exploration Although sampling around the local areas of high-fitness latent codes can guarantee the superiority of the generated sequences, the search process may be prone to be trapped in these local regions after a certain number of generations, thereby hindering the exploration of potentially promising sequences, which are unseen before. As a result, we randomly add white noise to the latent variables when $p \sim \mathcal{U}[0, 1)$ is higher than a threshold. As demonstrated in line 14 of Algorithm 1, the formula is defined as:

$$z_l = z + (\gamma - \delta g)\epsilon, \epsilon \sim \mathcal{N}(0, I), \tag{6}$$

where $\gamma \in \mathbb{R}$ denotes the step size that controls the exploration rate, and δ is the annealing factor at the generation g of the evolution process. We hypothesize that when g gets close to G, i.e., the total number of generations, the population tends to contain superior samples; thus, we slow down the exploration to avoid degeneration at the end of the algorithm.

D Experimental Setup

Table 2: Detailed information and statistics of the eight protein datasets.

Dataset	Organism	Protein	Optimization Target	Length	Size	Percentiles		
						0.25	0.50	0.75
avGFP [36]	Aequorea victoria	GFP	Brightness	237	51,715	1.428	3.287	3.161
AAV [7]	Homo sapiens	VP1	AAV viabilities	28	42,330	−3.964	−0.840	1.321
TEM [14]	Escherichia coli	TEM-1 β-Lactamase	Thermodynamic stability	286	5,199	0.049	0.444	0.934
E4B [43]	Mus musculus	UBE4B	Ubiquitin ligase activity	102	91,032	−1.830	−0.984	−0.093
AMIE [53]	Escherichia coli	Amidase	Hydrolysis activity	341	6,417	−1.228	−0.666	−0.263
LGK [25]	Lipomyces starkeyi	Levoglucosan kinase	Levoglucosan utilization	439	7,633	−0.871	−0.562	−0.394
Pab1 [31]	Saccharomyces cerevisiae	Poly(A)-binding	mRNA binding	75	36,389	−0.116	−0.022	0.036
UBE2I [50]	Homo sapiens	UBE2I	Growth rescue rate	159	3,022	0.068	0.492	0.766

Datasets Following [35] and [41], we assess the performance of our method across eight protein engineering benchmarks: (1) Green Fluorescent Protein (**avGFP**), (2) Adeno-Associated Viruses (**AAV**), (3) TEM-1 β-Lactamase (**TEM**), (4) Ubiquitination Factor Ube4b (**E4B**), (5) Aliphatic Amide Hydrolase (**AMIE**), (6) Levoglucosan Kinase (**LGK**), (7) Poly(A)-binding Protein (**Pab1**), (8) SUMO E2 Conjugase (**UBE2I**). The comprehensive dataset information, including the protein name, organism, optimization target, sequence length, data size, and data percentiles, is provided in Table 2. Each dataset represents distinct optimization target, which we simplify as a singular term *"fitness"* when reporting the benchmark results. Detailed descriptions of the data are provided in the Supplementary Material.

Implementation Details The model training is conducted using a single NVIDIA A100 card, employing the Adam optimization algorithm [22] with a learning rate of 2e-4. Each dataset is randomly split into training and validation sets at a ratio of 9:1. To control the disentanglement property in the latent representation, we adopt the strategy proposed by [39] and set the expected KL values to be 20. We train the VAE model for 130 epochs and choose the best checkpoint for later inference. The experiments are run five times, and the average scores are reported. For inference, we perform gradient ascent as the warm-up phase for $T = 500$ iterations with the learning rate $\alpha \in [0.001, 0.01]$. The latent-based directed evolution involves $G = 10$ iterative processes, with the candidate number set to $K \times B = 128$. In our implementation, we set the number of samples and beam size to $K = 128$ and $B = 1$, respectively. Finally, for the random exploration, we try multiple combinations of annealing factor $\delta = 0.1$ and exploration step size $\gamma \in [1.5, 6]$ and report the best outcomes.

Baseline Algorithms We compare our method against the following representative baselines: (1) **AdaLead** [40] is an advanced implementation of model-guided evolution. (2) **DyNA PPO** [1] applies proximal policy optimization to search sequences on a learned landscape model. (3) **CbAS** [5] is a

probabilistic modeling framework and uses an adaptive sampling algorithm. (4) **CMA-ES** [17] is a famous evolutionary search algorithm. (5) **COMs** [45] is conservative objective models for offline MBO. (6) **PEX** [35] is a model-guided sequence design algorithm using proximal exploration. (7) **GFN-AL** [19] applies GFlowNet to design biological sequences. (8) **GGS** [24] is a graph-based smoothing method to optimize protein sequences. To ensure precise evaluation, we re-execute and re-evaluate all baseline methods using the same oracle. For the implementation from (1) to (4), we employ the open-source implementation provided by [40]. Regarding other baseline methods, we utilize the codes released by their respective authors. We were unable to evaluate [41] due to unrunnable public code.

Oracles To ensure unbiased evaluation and avoid circular use of oracles, following [26], we use two separate oracles for each fitness dataset: (1) the optimization oracle that guides the model optimization and (2) the evaluation oracle that assesses the performance of the methods. Following [35], we freeze the ESM-based encoders and fine-tune an attention 1D decoder stacked after them to predict the fitness scores. For fair comparisons, we only train the optimization oracle with the pre-trained 33-layer ESM-2 as the encoder, while using the pre-trained evaluation oracle provided by [35] to assess our method and other baselines.

Evaluation Metrics We use three metrics defined in [18] to evaluate our method and compare with other baselines: (1) **MFS**: maximum fitness score, (2) **Diversity**, (3) **Novelty**. The metrics are computed as follows:

- **MFS** = $\max(\{\mathcal{E}(p_i)\}_{i=1}^{N})$,
- **Diversity** = $\dfrac{\sum_{i=1}^{N}\sum_{j=1,j\neq i}^{N} d(p_i,p_j)}{N(N-1)}$,
- **Novelty** = $\dfrac{\sum_{i=1}^{N}\min_{s_j\in\mathcal{D}} d(p_i,s_j)}{N}$,

where $d(\cdot,\cdot)$ is the Levenshtein distance, and $\mathcal{D}$ is the initial dataset (i.e., training dataset). It is crucial to emphasize that greater diversity and novelty do *not* equate to superior performance, but offer insights into the exploration and exploitation trade-offs exhibited by different methods.

E Additional Experimental Results

Table 3: Diversity across eight protein datasets. This table provides insight into the exploration and exploitation trade-off among methods.

Models	avGFP	AAV	TEM	E4B	AMIE	LGK	Pab1	UBE2I	Average
AdaLead	9.814	6.904	8.071	7.412	6.898	7.430	7.441	7.439	7.676
DyNA PPO	204.376	114.229	157.964	140.617	171.123	205.098	185.145	179.212	169.721
DbAS	205.717	115.426	159.524	142.044	172.539	206.819	186.761	180.758	171.199
CbAS	205.709	115.437	159.502	142.033	172.526	206.823	186.766	180.769	171.196
CMA-ES	173.365	96.882	134.633	119.388	145.151	174.274	157.098	152.058	144.106
COMs	70.319	45.761	73.191	68.398	100.731	126.623	115.242	109.650	88.739
PEX	7.048	4.782	6.692	6.625	6.388	7.031	7.012	7.219	6.600
GFN-AL	6.253	0.501	1.234	29.057	46.577	11.248	27.134	3.253	28.231
GGS	4.630	12.712	7.385	10.577	14.692	16.151	16.730	3.011	10.744
LDE (ours)	94.646	1.604	61.652	4.504	35.762	108.053	9.040	14.666	41.806

Table 4: Novelty across eight datasets. This table provides insight into the exploration and exploitation trade-off among methods.

Models	avGFP	AAV	TEM	E4B	AMIE	LGK	Pab1	UBE2I	Average
AdaLead	13.486	17.805	41.405	48.934	41.167	78.868	73.526	78.548	47.967
DyNA PPO	201.702	111.566	156.784	139.227	170.368	205.815	185.686	179.801	168.869
DbAS	201.838	111.544	156.858	139.222	170.066	205.439	185.366	179.545	168.735
CbAS	201.825	111.549	156.845	139.172	170.031	205.404	185.354	179.549	168.716
CMA-ES	202.155	111.467	156.968	139.126	157.701	193.991	175.414	170.746	163.446
COMs	184.177	98.831	111.931	101.930	123.590	150.657	134.298	129.795	129.401
PEX	4.323	1.930	4.461	4.748	3.031	8.614	4.356	4.779	4.530
GFN-AL	220.632	2.452	255.939	29.062	326.255	413.951	64.046	145.458	192.728
GGS	3.552	2.061	4.029	8.081	9.989	20.990	8.380	2.862	7.493
LDE (ours)	91.863	0.657	62.508	2.037	10.423	65.145	2.875	126.495	45.250