Population-Based Black-box Optimization for Biological Sequence Design

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Abstract

The use of black-box optimization for the design of new biological sequences is an emerging research area with potentially revolutionary impact. The cost and latency of wet-lab experiments requires methods that find good sequences in few experimental rounds of large batches of sequences — a setting that off-the-shelf black-box optimization methods are ill-equipped to handle. We find that the performance of existing methods varies drastically across optimization tasks, posing a significant obstacle to real-world applications. To improve robustness, we propose Population-Based Black-Box Optimization (P3BO), which generates batches of sequences by sampling from an ensemble of methods. The number of sequences sampled from any method is proportional to the quality of sequences it previously proposed, allowing P3BO to combine the strengths of individual methods while hedging against their innate brittleness. Adapting the hyper-parameters of each of the methods online using evolutionary optimization further improves performance. Through extensive experiments on in-silico optimization tasks, we show that P3BO outperforms any single method in its population, proposing higher quality sequences as well as more diverse batches. As such, P3BO and Adaptive-P3BO are a crucial step towards deploying ML to real-world sequence design.

1. Introduction

The ability to design new protein or DNA sequences with desired properties would revolutionize drug discovery, healthcare, and agriculture. However, this is a particularly challenging optimization problem: the space of sequences is discrete and exponentially large; evaluating the fitness of proposed sequences requires costly wet-lab experiments; and not just one but a diverse set of high-quality sequences must be discovered to improve the chance of a candidate surviving downstream screening (e.g., for toxicity).

The task is not insurmountable, however, due to modern experimental technology, where hundreds to thousands of sequences can be evaluated in parallel. This forms the basis for directed evolution, a form of human-guided local evolutionary search (Arnold, 1998), and several ML-based methods (Section 4). Additionally, development of ML methods suitable to guide sequence design can be aided by working in-silico, instead of relying on wet-lab processes during algorithmic development.

In this paper, we introduce several in-silico design problems upon which we evaluate such ML methods. Unfortunately, we find that popular optimization methods are particularly sensitive to hyper-parameter choice and can have strong inductive biases that allow them to excel at some problems but perform poorly on others (Figure 1). This lack of robustness is a serious concern for practical application of these methods, which could cause wet-lab experiments to fail. We further find that several existing methods are ill-suited to generating diverse batches of sequences. Instead of using the batch size efficiently, methods tend to generate very similar sequences.

To improve robustness and sequence diversity, we introduce Population-Based Black-box Optimization (P3BO). P3BO draws inspiration from portfolio algorithms (Leyton-Brown et al., 2003; Tang et al., 2014) for numerical optimization: instead of generating a batch of sequences using a single potentially brittle algorithm, P3BO samples sequences from a portfolio of algorithms, allocating budget to each algorithm based on the quality of its past proposed sequences.

To our knowledge, P3BO is the first approach to leverage an ensemble of optimizers for batched optimization, a crucial characteristic of wet-lab optimization loops. We show that batching offers a dimension along which ensembling yields significant gains.

Notably, samples acquired by one algorithm are shared with all other algorithms, allowing each of them to learn from data acquired by all. By combining the strengths of mul-
Multiple algorithms, P3BO hedges against the risk of choosing an unsuitable optimization method for the problem at hand (Section 2.2). Sampling sequences from multiple algorithms additionally allows P3BO to produce more diverse batches and find distinct optima faster. We employ a heterogeneous population, consisting of global model-based optimizers based on discriminative and generative models along with local search using evolutionary strategies. Finally, we further improve P3BO by introducing a variant, Adaptive-P3BO, which adapts the hyper-parameters of the algorithms themselves on the fly using evolutionary search.

We evaluate P3BO and Adaptive-P3BO empirically on over 100 batched black-box optimization problems, and show that P3BO and Adaptive-P3BO are considerably more robust, generate more diverse batches of sequences, and find distinct optima faster than any single method in their population. Adaptive-P3BO improves upon P3BO results, and furthermore is able to recover from a poor initial population of methods. Our contributions are as follows:

- We introduce new in-silico optimization problems for benchmarking biological sequence design methods.
- We evaluate state-of-the-art sequence design methods across these problems, bringing to light two significant shortcomings of existing methods: (a) lack of generalization across similar problem classes, and (b) sub-optimal use of the large batch sizes crucial to wet-lab settings.
- We introduce P3BO: a population-based optimization framework for discrete batched black-box function optimization that ensembles over algorithms to hedge against brittleness and improve diverse sequence discovery.
- We further extend P3BO to Adaptive-P3BO, which mutates its population of methods online using evolutionary search, yielding further improvements upon P3BO.

2. Problem Setting and Motivation

We define sequences as elements of $\mathcal{V}^L$, where $\mathcal{V}$ is a finite vocabulary (for DNA, $|\mathcal{V}| = 4$; for proteins, $|\mathcal{V}| = 20$) and $L$ is the sequence length. For variable length sequences, we assume that sequences are padded to length $L$ by an end-of-sequence token.

Sequence design aims to maximize a function $f : \mathcal{V}^L \to \mathbb{R}$, which can be evaluated on batches of size $B \gg 1$, but only a limited number of times $T$.

2.1. Algorithm Requirements

Most discrete black-box optimization methods have associated hyper-parameters. Throughout the paper, an algorithm $A$ refers to an instance of a particular method class (e.g., evolutionary search), which is instantiated by a specific hyper-parameter configuration (e.g., mutation rate for evolutionary search). As P3BO ensembles heterogeneous algorithms, including global model-based optimizers and local search strategies, we make the following assumptions about the interface of algorithms.

$A.\text{fit}(\mathcal{X}, \mathcal{Y})$ updates its internal state (e.g., ML model) using a batch of sequences $\mathcal{X}$ and their objective values $\mathcal{Y} = \{f(x) \mid x \in \mathcal{X}\}$. Next, $A.\text{propose()}$ suggests a single sequence to be evaluated next. We require that $\text{fit}(\mathcal{X}, \mathcal{Y})$ can leverage data obtained by other algorithms, which prohibits the use of on-policy RL methods.

2.2. Robustness of Black-Box Optimizers

Figure 1 demonstrates the lack of robustness of existing optimization methods (Section B) on two representative in-silico optimization problems (Section A). On PDBIsing (left), model-based optimization (MBO), an optimizer based on a discriminative model of $f(x)$, performs well, and DbAs-VAE, which employs a generative model, struggles. On PfamHMM, their relative performance reverses order.
We introduce P3BO, a robust black-box optimization method that constructs batches of sequences with which to query \( f(x) \) using a population of heterogeneous optimization algorithms. By sharing data between algorithms, algorithms benefit from each other’s distinct exploration strategies.

### 3.1. Method Summary

The high-level structure of P3BO is summarized in Algorithm 1 and Figure 2. The input is an initial population \( \mathcal{P}^0 = \{ A_1, \ldots, A_N \} \) of \( N \) algorithms, which can be sampled from a distribution over algorithms or chosen using prior knowledge. At experimental round \( t \), P3BO constructs a batch \( \mathcal{X}^t \) of \( B \) sequences by iteratively sampling algorithm \( A_i \) from a categorical distribution parameterized by \( p^i \), then using \( A_i \) to propose a sequence \( x \) to add to the batch.

Crucially, we build the batch incrementally in order to duplicate sequences on the fly. Measuring a batch of non-unique sequences in the wet lab would be a waste of experimental resources. In future work, it would be natural to extend propose\( () \) to take the partially-constructed batch as input, in order to improve the batch diversity.

P3BO weights algorithms proportional to the quality of sequences that they found in the past, sampling more sequences from algorithms that found higher quality sequences. This is done by computing a reward \( r_i^t \) for each algorithm \( A_i \) at each step \( t \) and adjusting the probability of sampling from \( A_i \) based on \( r_i^t \). To evaluate the performance of each algorithm in the population, P3BO keeps track of which algorithms proposed each sequence in \( \mathcal{X}^t \) using subsets \( \mathcal{X}^t_i \) and \( \mathcal{X}^t_j \). If two algorithms \( A_i \) and \( A_j \) propose the same sequence \( x \in \mathcal{X}^t \), \( x \) is added to both \( \mathcal{X}^t_i \) and \( \mathcal{X}^t_j \).

After generating batch \( \mathcal{X}^t \), the target function \( f(x) \) is evaluated, yielding observations \( \mathcal{Y}^t \). These are used to compute rewards for each method (Section 3.2), which are used in turn to compute sampling probabilities \( p^{i+1} \) for the next round. Finally, fit\( () \) is called for each algorithm in the new population on \( \langle \mathcal{X}, \mathcal{Y} \rangle \). The fitting step itself is algorithm-specific, and can entail time-consuming steps such as fitting discriminative or generative models. Some algorithms, such as evolutionary search, can only propose sequences if fit\( () \) has been called on a non-empty set of data. In this case, we uniformly sample from the search space in the first round.

### 3.2. Selecting from a Population of Algorithms

To generate high-quality sequences consistently across optimization rounds and different optimization problems, P3BO must adjust each constituent algorithm’s contribution over time. At round \( t \), P3BO observes all objective function values for sequences \( \mathcal{X}^t_i \) that were proposed by algorithm \( A_i \). These observations are converted into a per-algorithm reward \( r_i^t \). Doing so is challenging because the the qualities of the sequences from each algorithm are correlated, since the algorithms share observations between optimization rounds.

In our experiments, we used the improvement of \( f(x) \) relative to \( f_{\text{max}} = \max \{ f(x) \mid x \in \mathcal{X} \} \), the maximum of \( f(x) \)
Algorithm 1 P3BO

0: Input: Population $\mathcal{P} = \{A_1, \ldots, A_N\}$
0: Input: Softmax temperature $\tau > 0$.
0: Input: Initial sampling weights $p^1$
0: $X, Y = \emptyset, \emptyset$ [Sequences and labels]
0: for $t = 1$ to $T$ do
1: $X^t = \emptyset$
2: $X_1, \ldots, X_N = \emptyset, \ldots, \emptyset$
3: while $|X^t| \leq B$ do
4: $i \sim \text{Categorical}(p^t)$
5: $X^t \leftarrow X^t \cup \{x\}$ [Only add $x$ if novel]
6: $X^t_i \leftarrow X^t_i \cup \{x\}$ [Only add $x$ if novel]
7: $Y^t = \{f(x) \mid x \in X^t\}$
8: $X, Y \leftarrow X \cup X^t, Y \cup Y^t$
9: $r^t = \text{get_rewards}(X, Y, \{X^t\})$ [Eq. 1]
10: $s^t = \text{decayed_rewards}(r^t)$ [Eq. 2]
11: if Adaptive P3BO then
12: $p^{t+1} = \text{softmax}(s^t / \tau)$ [Eq. 3]
13: for $A_i \in \mathcal{P}$ do
14: $A_i, \text{fit}(X, Y)$
15: return $X = 0$

of all previous rounds:

$$r^t_i = \frac{\max\{f(x) \mid x \in X^t_i\} - f_{\text{max}}}{f_{\text{max}}}.$$  \hfill (1)

Future work should consider alternative reward functions to (1), such as novelty search (Lehman & Stanley, 2008).

As P3BO aims to address lack of robustness in existing methods, we prefer algorithms that consistently propose good sequences. To do so, the probability $p_i$ of sampling algorithm $A_i$ depends not only on its reward at time $t$ but also past rewards via a credit score $s^t_i$:

$$s^t_i = \sum_{t' \leq t} r^t_i \gamma^{t-t'},$$  \hfill (2)

$$p_i = \frac{\exp(s^t_i / \tau)}{\sum_j \exp(s^t_j / \tau)}.$$  \hfill (3)

The decay rate $\gamma$ trades-off past and present improvements, assigning higher credit scores to algorithms that improve $f(x)$ consistently across optimization rounds. $s_i$ are min-max normalized values of the credit scores, which ensures that the $p_i$ are independent of the scale of the rewards. The hyper-parameter $\tau$ controls the entropy of the distribution, effectively trading off the exploration and exploitation of algorithms. If $\tau$ is set to a high value, sequences are sampled uniformly from the population, regardless of their rewards.

3.3. Adaptive Population-Based Optimization

Although the credit assignment and selection strategy described in Section 3.2 increases robustness by sampling few or no sequences from poorly performing methods in the population, it is limited to the set of algorithms that are already in the population. For example, the hyper-parameters of algorithms in the population can be sub-optimal for a particular problem, which upper-bounds the performance of P3BO. We address this limitation by optimizing hyper-parameters of algorithms in the population online by evolutionary search (Algorithm 2).

We first select the set $S$ of algorithms with the top-$q$ credit scores from $\mathcal{P}$, where $q$ is a quantile cut-off. We then use tournament selection (Miller et al., 1995) to select $k = 2$ parent algorithms from the pool of survivors $S$, and recombine their hyper-parameters. If the parents belong to different classes of algorithms and their hyper-parameters are incompatible, we select one of them randomly. Otherwise, we cross-over their hyper-parameters with some crossover rate. Finally, we mutate the resulting hyper-parameters with some mutation rate by either resampling hyper-parameters values from a prior distribution, or scaling them by a constant.

Algorithm 2 Adaptation of population members

0: Input: Population of algorithms $\mathcal{P} = \{A_1, \ldots, A_N\}$
0: Input: Algorithm scores $s = \{s_1, \ldots, s_N\}$
0: Input: Quantile cutoff $q$
0: $S = \{A_i \in \mathcal{P} \mid f_i \geq q\}$
0: $\bar{\mathcal{P}}, \bar{s} = \emptyset, \emptyset$
0: for $i = 1$ to $N$ do
1: parents = tournament_select($S$)
2: $A_i, s_i = \text{recombine(parents)}$
3: $A_i = \text{mutate}(A_i)$
4: $\bar{\mathcal{P}} \leftarrow \bar{\mathcal{P}} \cup \{A_i\}$
5: $\bar{s} \leftarrow \bar{s} \cup \{s_i\}$
6: return $\bar{\mathcal{P}}, \bar{s} = 0$

4. Related Work

Our work draws from related research in both ML-guided sequence design and population based optimization.

**ML for Sequence Design.** Methods based on generative models seek to maximize the expected value $\mathbb{E}_{p(x)}[f(x)]$ of the objective function $f(x)$ when sampling sequences $x$ from a distribution $p(x)$ that is parameterized, for example, using a RNN. Most approaches for maximizing this expectation can be seen as instances of the cross-entropy method (De Boer et al., 2005; Neil et al., 2018; Brookes & Listgarten, 2018; Gupta & Zou, 2018; Brookes et al., 2019a). At each round, the distribution is trained to maximize the likelihood of high-reward sequences seen so far; the next batch is sampled from this distribution.
Throughout the paper, we use ‘model-based optimization’ to refer to machine learning approaches that employ a discriminative surrogate model \( f(x) \) that approximates \( f(x) \). The surrogate is converted into an acquisition function, which is optimized to propose the next batch of sequences (Hashimoto et al., 2018; Wang et al., 2019; Yang et al., 2019; de Jongh et al., 2019; Liu et al., 2019; Sample et al., 2019; Wu et al., 2019b; Biswas et al., 2020).

Approximating \( f(x) \) by a surrogate \( \hat{f}(x) \) has two advantages. First, \( \hat{f}(x) \) is inexpensive to evaluate unlike \( f(x) \), which can require costly wet-lab experiments. Second, knowledge of the structure of \( \hat{f}(x) \) or its gradients can be used to guide the optimization of the acquisition function. Existing methods differ in the form of \( \hat{f}(x) \), the type of the acquisition function (e.g., expected improvement (Mockus et al., 2014)), and how the acquisition function is optimized.

Recently, Angermueller et al. (2020) proposed a hybrid approach for sequence design, where a generative policy is updated using model-based RL if the surrogate discriminative model is accurate, and model-free RL otherwise.

**Population-Based Optimization.** Ensembling is a common strategy to produce robust algorithms by combining diverse algorithms that have individual weaknesses. Ensembles of evolutionary and swarm algorithms have been proposed for non-batched optimization, including multi-strategy methods (Du & Li, 2008), portfolio algorithms (Leyton-Brown et al., 2003; Tang et al., 2014), and hyper-heuristics (Burke et al., 2013). Existing approaches surveyed in Wu et al. (2019a) can be categorized into low-level ensembles, which agglomerate different instances of the same class of algorithm such as different mutation operators for evolutionary search, and high-level ensembles, which operate over algorithms belonging to heterogeneous families. P3BO belongs to the later category.

Ensemble optimizers differ in how constituent algorithms are selected over time, also known as adaptive operator selection (AOS) (Maturana et al., 2009; Fialho et al., 2010a; Li et al., 2013). AOS first defines the credit score of operators based on their past rewards and then selects operators based on their score. The average reward, relative reward improvement, or sum of ranks are common credit assignment strategies. Wu et al. (2019a) includes a review of such operator selection techniques, which tend to resemble the popular weighted majority method (Littlestone et al., 1989) for multi-armed bandits. AOS is not a bandit problem, however, since the action at time \( t \) impacts the rewards that different algorithms experience at later steps.

Evolutionary reinforcement learning (Pourchot & Sigaud, 2018; Khadka & Tumer, 2018) adapts a population of agents over time and exchanges observations between them. However, it is a low-level ensemble, combining homogeneous RL agents, and it performs non-batched, continuous optimization in the space of agents’ parameters. Evolutionary black-box optimization (PBT) (Jaderberg et al., 2017) jointly optimizes the weights and hyper-parameters of neural networks. However, the optimization is on a static training set, instead of data collected on-the-fly. AlphaStar applies population based training to evolve a set of agents in a multi-agent RL setting (Vinyals et al., 2019).

### 5. In-Silico Benchmarking Problems

Before deploying optimization methods on expensive wet-lab experiments, it is crucial to tune them using in-silico surrogates. This section describes a set of diverse problems that we have used to study the strengths and weaknesses of different methods. Further details about benchmark problems are described in Section A.

Creating realistic problems is challenging because protein fitness landscapes have not been experimentally well-characterized, and understanding their properties is an open research question. However, we can create a set of \( \hat{f}(x) \) with a range of functional forms containing elements that appear in real landscapes. We use: (1) exhaustive wet-lab measurements of all sequences in the search space for very short sequences, (2) regressors fit to wet-lab measurements for a subset of sequences from problems with larger search spaces, (3) neural networks with random weights, and (4) statistical models for protein sequence evolution fit using experimental data. Problems vary in the size of their search space, the number of initial samples provided to optimizers, and the sensitivity \( f(x) \) to shifts, insertions, and deletions of \( x \). See Section A for more details about individual optimization problems.

**TfBind8:** Barrera et al. (2016) measured the binding activity between a variety of human transcription factors and every possible length-8 DNA sequence. For each transcription factor, the optimization goal is to identify DNA sequences that maximize the binding activity score.

**TfBind10:** (Le et al., 2018) provides neural network predicted estimates of the relative binding affinities between all unique length-10 DNA sequences and each of two protein targets. For each target, the goal is to identify DNA sequences that maximize the predicted binding affinity.

**UTR:** Sample et al. (2019) introduced a CNN to predict the impact of a 5'UTR sequence on the expression level of its corresponding gene. The goal is to identify length-50 DNA sequences that maximize the predicted expression level.

**RandomMLP/RandomRNN:** Following Brookes & Listgarten (2018), we design optimization problems based on randomly initialized fully-connected and recurrent neural networks.
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### Table 1: Ranking of methods in terms of the area under the Max reward curve per optimization class (higher is better). Shown is the mean rank over all instances per optimization problem (Table 2). As also shown in Figure 3, both P3BO and Adaptive-P3BO outperform or are comparable to all other baseline methods for sequence optimization.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Adap. P3BO</th>
<th>P3BO</th>
<th>MBO</th>
<th>DbAs-VAE</th>
<th>LatentMBO</th>
<th>Evo</th>
<th>SMW</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDBIsing</td>
<td></td>
<td>7.0</td>
<td>5.6</td>
<td>5.2</td>
<td>3.8</td>
<td>3.0</td>
<td>2.3</td>
</tr>
<tr>
<td>PfamHMM</td>
<td>5.8</td>
<td>5.0</td>
<td>2.4</td>
<td>6.0</td>
<td>3.6</td>
<td>2.2</td>
<td>3.0</td>
</tr>
<tr>
<td>ProteinDist.</td>
<td>6.8</td>
<td>6.2</td>
<td>4.8</td>
<td>3.0</td>
<td>4.1</td>
<td>2.1</td>
<td>1.0</td>
</tr>
<tr>
<td>RandomMLP</td>
<td>7.0</td>
<td>5.9</td>
<td>4.5</td>
<td>3.6</td>
<td>3.9</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>RandomRNN</td>
<td>6.2</td>
<td>6.7</td>
<td>5.1</td>
<td>4.2</td>
<td>3.2</td>
<td>3.7</td>
<td>2.2</td>
</tr>
<tr>
<td>TiBind10</td>
<td>5.0</td>
<td>6.5</td>
<td>6.5</td>
<td>3.0</td>
<td>2.0</td>
<td>1.0</td>
<td>4.0</td>
</tr>
<tr>
<td>TiBind8</td>
<td>6.8</td>
<td>5.9</td>
<td>4.5</td>
<td>3.0</td>
<td>1.3</td>
<td>1.9</td>
<td>4.6</td>
</tr>
<tr>
<td>UTR</td>
<td>7.0</td>
<td>6.0</td>
<td>2.0</td>
<td>4.0</td>
<td>4.0</td>
<td>5.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

**Figure 3:** Ranking of methods in terms of the area under the Max reward curve per optimization problem (higher is better). Shown is the distribution of ranks over all instances per optimization problem (Table 2). The box of P3BO is flat since both the 25% and 75% percentile over all 105 optimization problems is 6.

PfamHMM: Pfam (El-Gebali et al., 2018) is a widely-used database of families of protein domain sequences. Each family consists of a small set of human-curated seed sequences, along with sequences that have been added automatically based on the likelihood under a profile hidden Markov model (HMM) fit using the seed sequences (Finn et al., 2011). We construct optimization problems for 24 diverse protein families. For each, we use the likelihood of the profile HMM as a black-box objective function. All optimization methods are provided with an initial dataset consisting of a random subset of the sequences in the family with likelihood in the bottom 50%. This simulates practitioners’ use of tools such as HMMer (Finn et al., 2011) or HHblits (Remmert et al., 2012) to find a set of evolutionarily-related sequences to inform sequence design.

ProteinDistance: Bileschi et al. (2019) introduced a CNN for protein domain classification that yields informative 1100-dimensional embeddings of protein domains. The ProteinDistance problem tasks methods to find sequences with high cosine similarity in the embedding space to representative sequences from Pfam families.

PDBIsing: As introduced in Angermueller et al. (2020), the goal of this problem is to find protein sequences that maximize the energy of an Ising model parameterized by a protein structure from the Protein Data Bank (Berman et al., 2003) (PDB). We reweight the objective function to place more emphasis on long-range pairwise interactions between amino acids. Optimization methods are provided with an initial dataset of mutants of the sequence that the PDB structure is based on.

6. Experiments

We next analyze the performance of a set of competitive optimization methods on the benchmark problems described in the previous section.

6.1. Baseline Optimization Methods

We consider a set of methods that have been designed for batched black-box optimization of biological sequences.

**SingleMutantWalker** (SMW) simulates site-saturation mutagenesis, where all single-mutation neighbors of the best sequence seen so far are proposed in a given optimization round (Wu et al., 2019b).

**Evolution** performs directed evolutionary search by selecting the top $k$ sequences, recombining them, and mutating them (unter Rudolph, 1958; Brindle, 1980).

**Cross-Entropy Method** fits a generative model to maximize the likelihood of high-quality sequences and samples the next batch of sequences from this model. **DbAs-VAE** uses the same example weighting scheme and generative model, a fully-connected variational autoencoder (Kingma & Welling, 2014), as in (Brookes & Listgarten, 2018). Supplementary material also considers FBGAN (Gupta & Zou, 2018), which uses a generative adversarial network.

**Model-Based Optimization** (MBO) explores a set of candidate regressor models by sweeping over model types and hyper-parameter values. All models with cross-validation performance above a predefined threshold are ensembled, yielding a predicted mean and variance for each sequence. These are converted into an acquisition function (e.g., expected improvement), which is optimized with regularized evolution (Real et al., 2019) to yield the next batch of sequences.

**Latent-Space MBO** (LatentMBO): We adapt the method of (Gómez-Bombarelli et al., 2018) to batched optimization. Model-based optimization is performed in the continuous latent space of a generative model that is trained jointly with a regressor model, which predicts $f(x)$ given the latent embedding of $x$. We use the cross entropy method for
optimizing the regressor.

6.2. Population-Based Optimization

We used a population with \( N = 15 \) constituent algorithms belonging to the following classes: SMW, MBO with various regressors, acquisition functions, and mutation rates of the evolution acquisition function optimizer; DbAs with different generative models (VAE or LSTM) and quantile thresholds; and Evolution, varying mutation and crossover probabilities. See Section B.5 for more details.

We sampled the initial population of algorithms randomly, ensuring that P3BO includes at least one instance per class to promote diversity, and at most four MBO instances to reduce computational costs. We used a decay factor of \( \gamma = 0.25 \) for computing credit scores, and a softmax temperature of \( \tau = 1.0 \) for computing selection probabilities.

We compare P3BO, which does not mutate inner algorithm hyper-parameters, to Adaptive-P3BO as described in Section 3.3. For Adaptive-P3BO, we set a quantile cutoff of \( q = 0.5 \) for selecting the pool of survivors \( S \), a recombination rate 0.1, and a mutation rate of 0.5. Experimentally, we observed that the performance of Adaptive-P3BO remained robust to any of these hyper-parameters.

6.3. Evaluation of Sample-Efficiency and Robustness

We evaluate sample-efficiency for a particular optimization problem by comparing the cumulative maximum of \( f(x) \) (Max reward) depending on the number of samples proposed. We further use the area under the Max reward curve for expression sample-efficiency in a single number and comparing methods across optimization problems. We repeat each experiment 20 times with different random seeds.

Figure 4 illustrates the mean optimization trajectories of P3BO, Adaptive-P3BO, and baselines over 6 different problem classes. P3BO and Adaptive-P3BO systematically find high-reward sequences faster than any baseline method, which shows that population-based optimization can increase both sample-efficiency and robustness. Adapting the population of optimizers (Adaptive-P3BO) yields further performance improvements. Non-population-based methods have inconsistent performance: there is no best non-population-based method across problems. Optimization trajectories for additional problem classes and baseline methods are shown in Figure 9 and Figure 10, respectively.

Table 1 and Figure 3 summarize results, which report the average ranking of different optimization methods across all 105 optimization problems. P3BO and Adaptive-P3BO are systematically more sample-efficient than any baseline method.
Figure 5: Insights into Adaptive-P3BO applied to PfamHMM target PF16186. Shown are the credit score (left), the number of sequences sampled (middle), and the number of instances (right) per algorithm class during the optimization trajectory. Since DbAs-VAE has the highest credit score (relative improvement) in early rounds, more sequences are sampled from DbAs-VAE (middle), and Adaptive-P3BO increases the number of DbAs-VAE instances from 4 to 11 (the total population size is 15). The adaptation starts after three warm-up rounds used to reliably estimate the credit score of algorithms. See Figure 11 for another example.

Figure 6: t-SNE plots of sequences found by methods in different rounds (a-c), and of all sequences with a reward greater than the 75th percentile of rewards found by all methods (d). Results are for TiBind8, targeted CRX_R90W_R1. SMW, Evo, and DbAs-VAE increasingly focus on a single search region, whereas MBO, P3BO and Adaptive-P3BO maintain a diversity of sequences within each batch (Fig. a-c). When showing only sequences that achieved a relatively high reward in Figure 6(d), we see that P3BO and Adaptive-P3BO still find diverse clusters of high-reward sequences; in fact, P3BO and Adaptive-P3BO find sequences in nearly all clusters identified by ensemble-free methods.

6.4. Evaluation of Diversity

We evaluate the ability of methods to find distinct sequences of high reward using the metrics summarized in Section C. Figure 7 reports on of these metrics—the fraction of optima found by each method on the TiBind8 problem. P3BO and Adaptive-P3BO find more optima than the best individual method (MBO), and considerably more optima than all remaining methods. Figure 8 further shows the mean distance of sequences in proposed batches, and the number of distinct clusters of sequences with a high reward that were found by methods. These results confirm that P3BO and Adaptive-P3BO find diverse sequences with a high reward across optimization problems.

Figure 6 uses t-SNE to illustrate the diversity of sequences obtained over time and at the end of optimization for each method. P3BO and Adaptive-P3BO not only find more diverse sequences per batch during the optimization process,
but also obtain diverse and high-reward sequences at the end of the optimization process, fulfilling a crucial requirement for real-world sequence validation.

6.5. Ablation Experiments

We perform ablation experiments to better understand the behavior of P3BO and Adaptive-P3BO.

Figure 5 illustrates how the scoring of algorithms affects the number of sequences sampled from each algorithm class and the number of instances per class for one PfamHMM problem. Adaptive-P3BO correctly identifies DAS-VAE as the best performing algorithm (see Figure 4, PfamHMM), and hence samples more sequences from DAS-VAE and enriches for the number of DAS-VAE instances in the population. We observe the same behavior on other optimization problems (Figure 11).

Figure 12(a) analyzes the performance of P3BO when removing individual algorithm classes from its population. As expected, the performance of P3BO is most sensitive to the best performing algorithm in its population. However, since the performance of algorithms varies across problems, removing individual algorithms results in a performance drop on some problems but not on others. Figure 12 also shows that sharing data between optimization methods enables poorly performing algorithms to learn from the sequences found by well performing algorithms and to find high-quality sequences faster, which results in an overall performance gain of P3BO. This is confirmed visually by Figure 13, which shows the t-SNE visualization of sequences proposed by individual algorithms over time with and without data sharing.

Figure 14 highlights that Adaptive-P3BO recovers faster than P3BO when starting with a poorly initialized population of methods, which it can improve online by evolutionary search as described in Section 3.3.

Finally, we investigate the sensitivity of P3BO to the choice of the scoring function (Figure 15, 16), the temperature $\tau$ of the softmax scoring function (Figure 17), the population size (Figure 18), and the batch size of the optimization problem (Figure 19).

7. Summary

We introduced P3BO and Adaptive-P3BO, ensemble-based approaches for black-box function optimization. P3BO and Adaptive-P3BO achieve both robustness across problems and a strong diversity of high-quality sequences, establishing them as a promising methodology for directing biological sequence design.
Population-Based Black-Box Optimization for Biological Sequence Design

References


Brindle, A. Genetic algorithms for function optimization. 1980.


### A. In-Silico Design problems

<table>
<thead>
<tr>
<th>Problem</th>
<th>Num Inst.</th>
<th>Num Rounds</th>
<th>Batch Size</th>
<th>Vocab Size</th>
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</table>

Table 2: Information about benchmark problems, including the number of instances per problem, the number of optimization rounds, batch size, vocabulary size, sequence length, and the number of initial samples. RandomMLP and RandomRNN considers sequences of length 20 or 40. The sequence length of PfamHMM and ProteinDistance varies between 50 and 100. The PfamHMM problem provides methods with 500 initial samples. All other problems do not provide initial samples.

Table 2 summarizes the considered optimization problems. For each problem, we construct multiple instances by varying the protein target (PDBIsing, PfamHMM, TfBind), or the neural network architecture and random seed for weight initialization (RandomMLP/RandomRNN). We provide additional details per problem in the following sub-sections.

#### A.1. TfBind

For a given transcription factor, the optimization goal is to produce length-8 DNA sequences that maximize the affinity of DNA-binding. We use 12 optimization instances, corresponding to the following transcription factors: CRX_REF_R1, CRX_R90W_R1, NR1H4_REF_R1, NR1H4_C144R_R1, HOXD13_REF_R1, HOXD13_Q325R_R1, GFI1B_REF_R1, FOXC1_REF_R1, PAX4_REF_R1, PAX4_REF_R2, POU6F2_REF_R1, and SIX6_REF_R1.

We min-max normalize the binding affinity values for each transcription factor target to the zero-one interval.

TfBind10 differs from TfBind8 in that sequences are of length 10 and only two transcription factors (Cbf1 and Pho4) are available, which were characterized experimentally (Le et al., 2018).

#### A.2. Random Neural Network

The goal is to optimize the scalar output of a randomly initialize neural network. Optimization proceeds over 10 rounds with batch size 100. We construct different instances by varying the sequence length (20 or 40), the vocabulary size (4 or 20), the random seed (0 or 13), and the architecture of the network (described in the following).

RandomMLP considers networks with a varying number of convolutional and fully connected layers. Networks have either no convolutional layer, or one layer with 128 units, a kernel width of 13, and a stride size of 1. The number of fully connected layers is either 1 (128 hidden units) or 3 (128, 256, 512 hidden units).

RandomRNN considers networks with 1 (128 hidden units), 2 (128, 256 hidden units), or 3 (128, 256, 512 hidden units) LSTM layers.

#### A.3. PfamHMM

Sequences annotated by Pfam within each family have variable length and the likelihood under the HMM can be evaluated for arbitrary-length sequences. For simplicity, however, here we consider optimization over fixed-length sequences. The length is chosen as the median length of unaligned sequence domains that belong to the Pfam-full sequence alignment for the corresponding family.

The initial dataset is obtained by (1) selecting all sequences from Pfam-full that belong to the given family and have the chosen length, (2) evaluating their likelihood under the HMM, and (3) sampling from the bottom 50%.

We selected families relatively short sequences. Future work might consider longer sequences while optimizing only a subset of positions.
A.4. ProteinDistance

The ProteinDistance problem tasks methods to find sequences with a high cosine similarity in the embedding space to a chosen target sequence. We used the network from Bileschi et al. (2019) to obtain embeddings of proposed sequences. This network was trained to classify the Pfam family of a protein sequence, and its embeddings have been shown to capture broad protein features that are useful for few-shot learning. We construct optimization problems by choosing different Pfam HMM seed sequences as the target sequence, and used the same Pfam families as in the PfamHMM problem.

A.5. PDBIsingModel

We employ $f(x) = \sum_i \phi_i(x_i) + \beta \sum_{i,j} C_{ij} \phi(x_i, x_j)$, where $x_i$ refers to the character in the $i$-th position of sequence $x$. $C_{ij}$ is a binary contact map dictating which positions are in contact with each other and $\phi(x_i, x_j)$ is a position-independent coupling block. We construct optimization problems by choosing 10 different proteins from the Protein Data Bank (Berman et al., 2003). $C_{ij} = 1$ if the $C\alpha$ atoms of the amino acid residues at positions $i$ and $j$ are separated by less than 8 Angstroms in the protein’s 3D structure. The coupling block is based on global co-occurrence probabilities of contacting residues (Miyazawa & Jernigan, 1996). The local terms $\phi_i(x_i)$ are set using the sequence of amino acids for the true PDB protein. The score for setting $x_i$ to a specified value is given by the log Blosum substitution probability between that value and the corresponding value in the PDB sequence. Finally, $\beta$ is chosen heuristically such that the local terms do not dominate the objective too much.

B. Optimization Methods

B.1. Model-Based Optimization

We follow (Angermueller et al., 2020) for building the regressor model. We optimize the hyper-parameters of diverse regressor models by randomized search, and evaluate model performance by the five-fold cross validation $R^2$ score. We select all models with a score $\geq 0.4$ and build an ensemble by averaging their predictions. We consider the following scikit-learn (Pedregosa et al., 2011) regressor classes and hyper-parameters:

- BayesianRidge: alpha_1, alpha_2, lambda_1, lambda_2
- RandomForestRegressor: max_depth, max_features, n_estimators
- LassoRegressor: alpha
- GaussianProcessRegressor: kernel (RBF, RationalQuadratic, Matern) and kernel parameters

We used the posterior mean acquisition function, which performed as good or better as the upper confidence bound, expected improvement, or probability of improvement. We optimize the acquisition function using evolutionary search for 500 rounds with a batch size of 25. We construct the next batch by selecting the top $n$ unique and novel sequences with the highest acquisition function value.

B.2. Latent-Space Model-Based Optimization

An alternative approach to discrete optimization is to train an encoder-decoder model that enables mapping discrete sequences to and from continuous vectors and moving the optimization process into the continuous space (Gómez-Bombarelli et al., 2018; Kusner et al., 2017; Roeder et al., 2018; Killoran et al., 2017; Cao et al., 2019; Luo et al., 2018; Gupta & Kundaje, 2019). The approach has been introduced for problems with a large amount of (unlabeled) initial data and a single round of optimization, and we adapt it for multi-round optimization. In each round, we use all the observed sequence-reward pairs to jointly train a variational auto-encoder (VAE) (Kingma & Welling, 2014) and a neural network regressor from latent embeddings to the corresponding rewards. Jointly training the regressor and VAE encourages organizing latent representations by reward scores (Gómez-Bombarelli et al., 2018). Once the encoder-decoder model has been fixed, similar to the previous work, we train a new regressor from scratch on the embedding-reward pairs, and use it to score new sequences during continuous optimization.

We train the VAE by maximizing the variational lower-bound (Kingma & Welling, 2014). We anneal the KL divergence and the neural network regressor loss over time. We standard scale regressor target labels, and use the mean-squared error as training objective. The encoder and decoder architectures match those in the DbAs-VAE; the regressor is a fully-connected network with a single hidden layer. We consider the following hyper-parameters (actual values in the parentheses): latent space size (50), learning rate (0.006), weight of the regressor loss term (2 * sequence length), sigmoid annealing slope (1.), batch size (20), number of epochs (60), hidden sizes for the encoder (128), decoder (128), and regressor (50). We use the same hyper-parameters across all LatentMBO rounds and we warm-start the VAE and regressor parameters with those from the preceding round.

For training a new regressor on the embedding-reward pairs, we use the ensemble-based approach described Section B.1. We perform optimization in the latent space using the cross entropy method with a diagonal multivariate Gaussian generative model. We perform weighted maximum likelihood on high scoring samples, re-weighted by the likelihood ratio of a given prior and the current generative model (CbAs) (Brookes et al., 2019b). We use as prior a diagonal multivariate Gaussian generative model fit on the training data of...
the VAE and regressor. The re-weighting approach encourages the optimization trajectory to remain close to the prior, which is desirable as the latent regressor performance is expected to degrade as we move away from training samples.

We run the cross entropy method for a fixed number of iterations. The proposed batch is obtained by decoding the best-scoring unique samples (according to the regressor score) across multiple runs (instances) of the cross entropy method with different random seeds. We tune the following hyper-parameters (actual values in the parentheses): the number of cross entropy method instances (25), the number of iterations per instance (20), the number of samples drawn per iteration (100), and the number high-scoring samples to extract (10). We initialize the cross entropy method with the embeddings of the highest scoring 25 sequences observed during the optimization. Finally, we note that, as optimization is being performed in the continuous space, one could use a differentiable regressor and gradient-based optimization. We chose the cross entropy method approach as, by construction, it provides a set of good candidates rather than a single one (Brookes & Listgarten, 2018; Brookes et al., 2019b).

B.3. Evolution:

In each round the evolution search approach generates a single sample by selecting two parents, recombining them, and mutating the child. Following (Real et al., 2019), samples are no longer considered during parent selection after a fixed number optimization rounds to prevent elite samples from dominating the population (“death by old age”). Two parents are chosen for each child via tournament selection, which involves taking the best of T samples from the alive population. The chosen parent sequences A and B are recombined by copying the sequences from left to right beginning with a pointer on parent A. At each position, the pointer has some probability (we used 0.1) of switching to reading the other parent. After crossover, we mutate the child by changing each position to a different value with a fixed mutation probability (we used 0.01).

B.4. Feedback GAN

We use a quantile cutoff for selecting the threshold for positive sequences, as this performs better than using a fixed \( f(x) \) threshold. We tuned the quantile cutoff, learning rate, batch size, discriminator and generator training epochs, the gradient penalty weight, the Gumble softmax temperature, and the number of latent variables of the generator.

B.5. P3BO

We set the population size of 15, and note that similar performance can be achieved with a smaller, but greater than 5, population size (see Figure 18). We selected MBO, DbAs-VAE, DbAs-RNN, Evolution, and SMW as a representative set of baseline methods to act as constituent algorithm classes (see Sec 6.1 in the main text for descriptions of these methods). We sampled hyper-parameters from following distributions:

**SMW** This method is hyper-parameter free.

**Evolution**

- crossover_probability: uniform(interval(0.1, 0.3))
- mutation_probability: uniform(interval(0.05, 0.2))

**DbAs-VAE**

- quantile: uniform(0.825, 0.975)
- learning_rate: loguniform(interval(0.008, 0.012))
- num_vae_units: uniform(categorical(64, 128))

**DbAs-RNN**

- quantile: uniform(0.9, 0.975)
- learning_rate: loguniform(interval(0.0005, 0.012))
- num_lstm_units: uniform(categorical(64, 128))

**MBO**

- acquisition_function(uniform(categorical([‘PosteriorMean’, ‘UCB’])))
- ucb_scale_factor: uniform(interval(0.5, 1.2))
- regressor: uniform(categorical([‘Ensemble’, ‘BayesianRidge’]))

Here, ‘Ensemble’ refers to the ensemble model described in B.1 and ‘BayesianRidge’ to the BayesianRidge regressor of the scikit-learn package.

C. Diversity metrics

Finding not only one high reward sequence but multiple diverse high reward sequences is desirable to increase the chance that some of the found sequences satisfy downstream screening criteria that are not captured by the primary optimization objective, e.g. stability or viscosity. For quantifying diversity, we devised the following metrics:

**Mean pairwise Hamming distance** We compute the mean pairwise Hamming distance of sequence in a batch. We also considered the Edit distance but found it to be too slow to compute for problems with large batch sizes.

**Mean entropy** For a given batch of sequences, we compute the entropy over characters at each position, and then average over positions. Since we found this metric to be highly correlated with the mean pairwise Hamming distance (\( R^2 = 0.99 \)), we are showing results only for the mean pairwise Hamming distance.
Number of clusters above a reward threshold  
For problems with known maximum we first select all sequences proposed so far with a reward above a threshold, e.g. $0.8 \cdot \max f(x)$. We then cluster the selected sequences using hierarchical complete linkage clustering, and count the number of resulting clusters with a minimum Hamming distance of 0.5. This metric can be maximized by finding well separated and high reward sequences.

Reward vs. Hamming distance  
We plot the mean reward of sequences in a batch vs. the mean pairwise Hamming distance, which shows the trade-off of these two metrics.

Fraction of optima found  
This metric can be only computed for enumerative optimization problems, in our case TfBind8. In a pre-computation step, we first select all possible sequences with a reward $\geq 0.9$. We then cluster selected sequences into distinct clusters with a minimum distance of 3 using hierarchical complete linkage clustering. We define the sequence with the highest reward of each cluster as one optimum. We count an optimum as found if the exact same sequence is proposed. We treat the forward and reverse sequence as two distinct optima. This metric differs from the 'Number of clusters’ metric in two points: 1) the number of clusters is normalized by the total number of clusters, and 2) the Edit distance is used for clustering sequences to assign sequences that contain similar motifs at different positions to the same cluster.

D. Additional results
Figure 8: Visualization of alternative diversity (Section C) for the PdbIsing, ProteinDistance, TIBind8, and UTR problem. We find that DbAs-VAE and Evolution generate more diverse sequences than P3BO, albeit with a lower reward. P3BO finds significantly more diverse and higher reward sequences than both MBO and SMW, and provides overall a good trade-off between finding high reward and diverse sequences.
Figure 9: Performance curves for the RandomMLP and TFBind10 problem, which were not shown in the main text due to space limitations. Lines show the average over all targets available for each problem, while the shaded areas indicate bootstrap-based 95% confidence-intervals.

Figure 10: Performances curves for different problem classes. This figure includes FBGAN and DbAs-RNN that performed worse than all other baselines methods and were therefore not shown in the main text figure. Lines show the average over all targets available for each problem, while the shaded areas indicate bootstrap-based 95% confidence-intervals.
Figure 11: Insights into Adaptive-P3BO applied to the UTR problem. Shown are the credit score (left), the number of sequences sampled (middle), and the number of instances (right) per algorithm class during the optimization trajectory. Since Evolution has the highest credit score (relative improvement) for early rounds, more sequences are sampled from Evolution (middle), and Adaptive-P3BO increases the number of Evolution instances from 4 to 11 (the total population size is 15). The adaptation starts after three warm-up rounds used to reliably estimate the credit score of algorithms.
Figure 12: Performance of P3BO on the PdbIsing, PfamHMM, and UTR problem when removing individual method classes from its population. The top row compares P3BO with all method classes (P3BO full) to variants with one class removed. The middle row shows the performance of methods when used stand-alone without data sharing, and the bottom row when used inside P3BO with data sharing. Removing MBO from the population of P3BO results in a performance drop on PdbIsing (top row) due to the good performance of MBO on that problem (middle row). In contrast, DbAs-VAE is the best performing method on PfamHMM, which results in a performance drop when removing it. Sharing samples acquired by one method with all other methods in the population (bottom row) results in a higher performance of individual methods than without sharing samples (middle row). For example, SMW benefits from the high-reward sequences found by MBO on PdbIsing in early rounds (middle row, left plot) and thereby manages to find sequences with a higher reward than MBO in following rounds (bottom row, left plot).
Figure 13: $t$-SNE of sequences proposed by different methods with and without sharing samples $(x, f(x))$ between methods. The shape of each point (sequence) corresponds to the method that proposed the sequence $x$ and the color to the reward $f(x)$. Without sharing samples $(x, f(x))$, methods propose distinct, well separated, sequences, and only MBO finds high reward sequences quickly. By sharing samples, methods benefit from the high reward sequences discovered by MBO in early rounds and propose similar sequences in subsequent rounds. Results are shown for PdbIsing model PDB ID 1KDQ.
Figure 14: Performance comparison between P3BO and Adaptive-P3BO when starting with a poorly initialized population of algorithms. By adapting the population online, Adaptive-P3BO can recover from the initial population, resulting in a clear performance improvement.

Figure 15: Comparison of the relative improvement reward function described in Section 3.2 with the rank-based reward function as proposed by Fialho et al. (2010b). Both approaches perform similarly across problems.
Figure 16: Performance of P3BO and Adaptive-P3BO when using the relative improvement scoring function described in Section 3.2 vs. scoring methods randomly.

Figure 17: Sensitivity of P3BO to the softmax temperature $\tau$ for computing selection probabilities (Section 3.2) on the PfamHMM, ProteinDistance, and UTR problem. Shows that scaling the number of sequences sampled from algorithms in the population proportional to their credit score ($\tau < 10$) is better than sampling sequences uniformly ($\tau \geq 10$).
Figure 18: Cumulative maximum of P3BO for different population sizes $N$ on three optimization problems. While a population size of 15 is best on average, similar performances can also be achieved smaller populations. Using only one algorithm ($N = 1$) results in a clear performance decrease. For this analysis, the initial population was sampled randomly from a pool of algorithms as described in Section 6.2.

Figure 19: Relative performance of methods depending on the number of samples per optimization round (batch size). The y-axis corresponds to the area under the cumulative max reward curve, min-max normalized across methods. Error bars show the variation across five optimization problems (one instance of the ProteinDistance, PfamHMM, UTR, and RandomMLP problem). Shows that P3BO is applicable to optimization settings with various batch sizes, including non-batched optimization.