Qbio Journal Club - mRNA Design

via Expected Partition Function and Continuous Optimization

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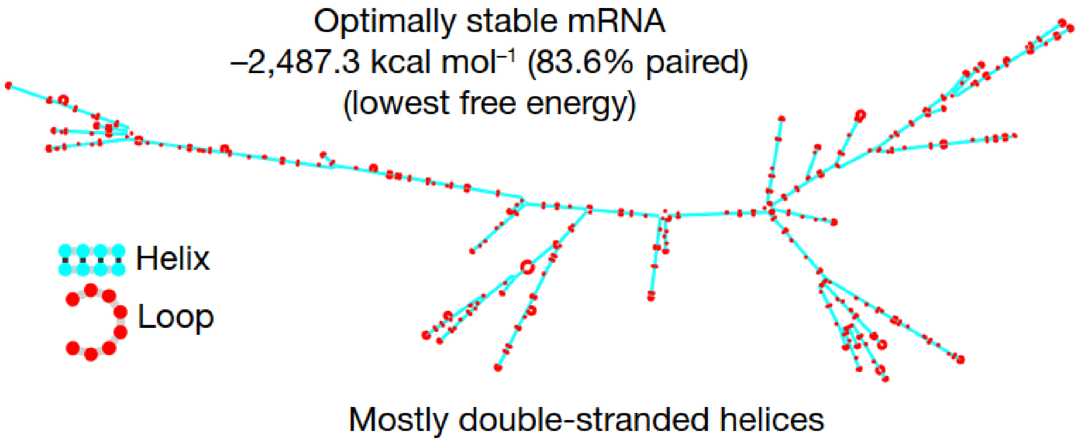
1. Introduction

1. Introduction

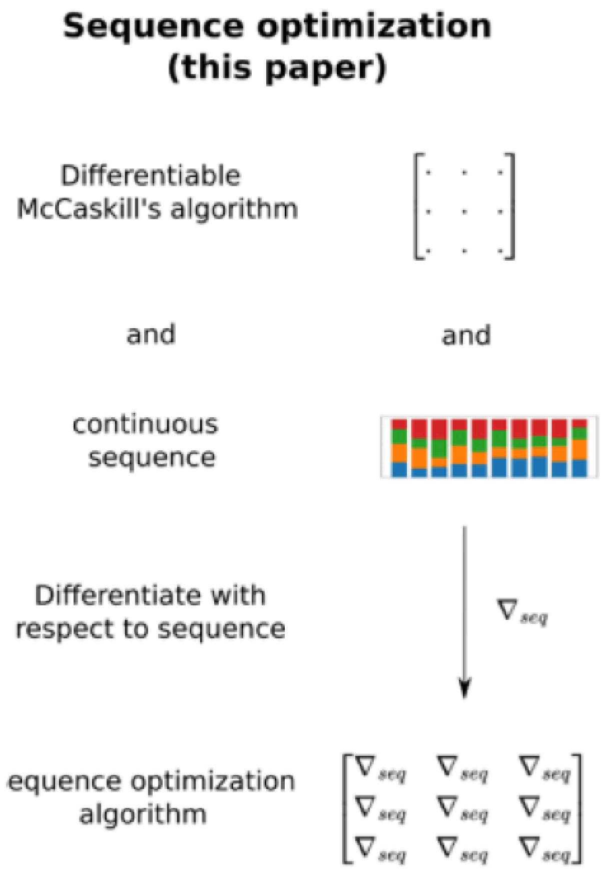
- RNA design can be categorized into two types:
  - **Design of Optimal Codon Sequence** for a given protein, in terms of factors such as stability and translatability. *LinearDeisgn* falls into this category. However, LinearDesign considers only the MFE and does not consider the entire ensemble of structures.
  - **Design of Optimal non-coding RNA** for a given function, which aims to find the RNA sequence that naturally folds into a desired structure. *Infrared* falls into this category.


Optimally stable mRNA
–2,487.3 kcal mol^{-1} (83.6% paired)
(lowest free energy)
Helix
Loop
Mostly double-stranded helices

Zhang H., et al. Nature 621.7978 (2023): 396-403.

1. Introduction

- The authors seek to translate the mRNA design problem from one of *discrete* optimization to one of *continuous* optimization.
- Take a probability distribution $\mathcal{S}$ os sequences. $\mathcal{S}$ can be expressed as a $L \times 4$ matrix with the element each row corresponding to the probability of each nucleotide.
- Define the expected partition $\overline{\mathcal{Z}}$ over $\mathcal{S}$. Using gradient descent over $\mathcal{S}$, $\mathcal{S} \to \mathcal{S}^*$, with S^* a one-hot encoding of the optimal sequence.
- The authors apply this algorithm to achieve **ensemble free energy (EFE)** lower than LinearDesign.



Matthies, M. C., et al. Nucleic Acids Research 52.3 (2024) e14-e14.

2. Expected Partition Function

2. Expected Partition Function

- An RNA sequence $\mathbf{x} = (x_1, x_2, \cdots, x_L)$ is a string of L nucleotides, where $x_i \in \{A, C, G, U\}$.

The Probabilistic Representation for an RNA sequence:

$\mathbf{x}$ can be represented as a sample from a probability distribution $\mathcal{S}$ written as a $L \times 4$ matrix.

· with constraints $S > 0$ and $S \times \mathbf{1}_4 = \mathbf{1}_L$, where $\mathbf{1}_n$ is the n-th dimensional vector of ones.

· where the probability of each nucleotide is assumed to be independent.

$$\mathcal{S} = \begin{bmatrix} p_{1A} & p_{1C} & p_{1G} & p_{1U} \\ p_{2A} & p_{2C} & p_{2G} & p_{2U} \\ \vdots & \vdots & \vdots & \vdots \\ p_{LA} & p_{LC} & p_{LG} & p_{LU} \end{bmatrix} \tag{2.1}$$

2. Expected Partition Function

- Having established a probability distribution $\mathcal{S}$, the authors define the **Expected Partition Function** $\overline{\mathcal{Z}}$ as the expected value of the partition function $\mathcal{Z}$.
- The partition function Z for a specific sequence $\mathbf{x}$ is defined as the sum of the Boltzmann factors over all possible secondary structures y where $y \in \{(,),.\}^L$.

$$\mathcal{Z}(\mathbf{x}) = \sum_{y \in \mathcal{Y}} \exp(-\beta G(\mathbf{x}, y)) \tag{2.2}$$

- with G the Gibbs Free Energy, $\beta = 1/kT$ the thermodynamic coldness, and $\mathcal{Y}$ the set of all valid secondary structures of $\mathbf{x}$.
- Eq. (2.2) can be extended using Eq (2.1) to define $\overline{\mathcal{Z}}$ as follows:

$$\begin{aligned}\overline{\mathcal{Z}}(\mathcal{S}) = \mathbb{E}_{x\sim S}[\mathcal{Z}(x)] &= \mathbb{E}_{x\sim S}\left[\sum_{y \in \mathcal{Y}} \exp(-\beta G(\mathbf{x}, y))\right] \\ &= \sum_{y \in \mathcal{Y}} \sum_{x \in \mathcal{S}} \prod_{1 \le i \le L} p_{ix_i} \cdot \exp(-\beta G(\mathbf{x}, y)) \end{aligned} \tag{2.3}$$

2. Expected Partition Function

- The authors provide the following pseudocode for the calculation of $\overline{\mathcal{Z}}$ using the Nussinov model
- The Nussinov model is a simplified model for approximating the energy of a secondary structure.
- It depends only on 20 parameters:
  - $\delta = (\delta_A, \delta_C, \delta_G, \delta_U)$
  - $\xi = \begin{pmatrix} \xi_{AA} & \xi_{AC} & \xi_{AG} & \xi_{AU} \\ \xi_{CA} & \xi_{CC} & \xi_{CG} & \xi_{CU} \\ \xi_{GA} & \xi_{GC} & \xi_{GG} & \xi_{GU} \\ \xi_{UA} & \xi_{UC} & \xi_{UG} & \xi_{UU} \end{pmatrix}$
- Translating the Nussinov model to the Turner model is a non-trivial task and is not covered by the authors, nor implemented in their current code↗. Memory↗ might be the bottleneck for a practical implementation of the Turner model.

2. Expected Partition Function

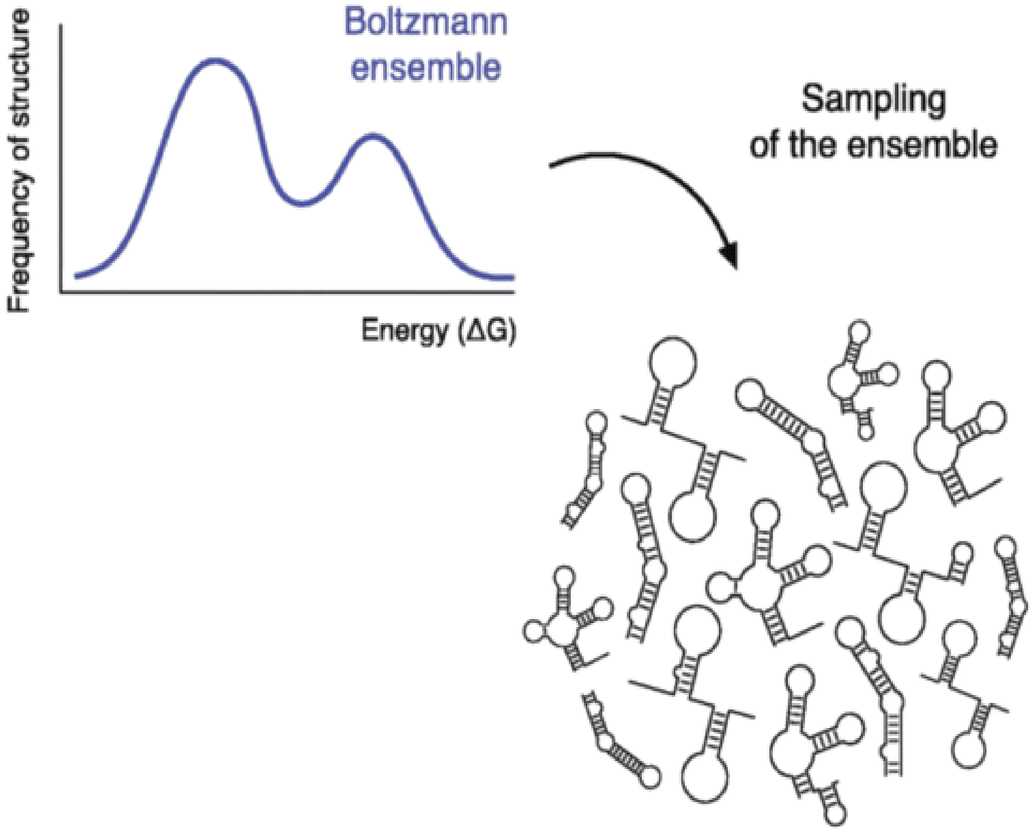
```python
def ExPartition(S):
    L = S.shape[0]
    S_pair = np.einsum('ik,jl->ijlk', S, S)
    Z = np.eye(N = L, k = -1)
    for j in range(L):
        Z[:, j] += Z[:, j - 1] * (S[j, :] @ np.exp(-BETA * DELTA).T)
        Z[:, j] += (Z[:, :-2] @ Z[2:, j-1]) * np.sum(
          S_pair[1: L - 1, j, :, :] * np.exp(
            - BETA * XI
          ))
    return Z
```

- The code has $\mathcal{O}(L^3)$ time complexity and $\mathcal{O}(L^2)$ space complexity. Applying beam search to the search space reduces the time complexity to $\mathcal{O}(Lb^2)$.

3. mRNA Design Problem

3. mRNA Design Problem

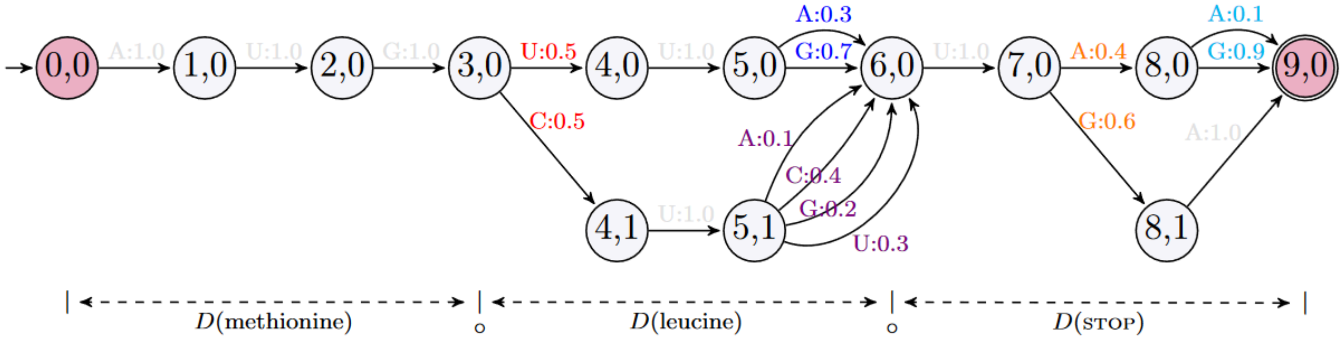
- The limitation of *LinearDesign* was the fact that it only considered the $\textbf{MFE} = \min_{y \in \mathcal{Y}} \Delta G(y)$.
- While the MFE is the most stable structure, there are multiple quasi-stable structures that are not considered in LinearDesign.
- The authors propose maximizing the Ensemble Free Energy (EFE) as a more accurate measure of stability. From thermodynamics, it is known that $\textbf{EFE}(\mathcal{S}) = \mathbb{E}_{s \sim \mathcal{S}, y \sim \mathcal{Y}} \Delta G(s, y) = -kT \log \overline{\mathcal{Z}}$.
- Thus minimizing the EFE is equivalent to maximizing $\overline{\mathcal{Z}}$.



Aviran S. Journal of Molecular Biology 434.18 (2022): 167635.

3. mRNA Design Problem

- To apply gradient descent over S in mRNA design problem, we need to define a way to transform $\mathbf{aa} = (aa_1, aa_2, \cdots, aa_L)$, a string of L amino acids, to $\mathcal{S}$, a distribution over sequences.
- The representation cannot be done by means of a matrix, as the probability of nucleotide at each position is not independent d/t degenerate amino-acids, e.g. Arginine and Leucine, and STOP.
- $\mathcal{S}$ is represented by a probabilistic DFA (Discrete Finite Automata), à la LinearDesign .
- In this representation of $\mathcal{S}$, each node is associated with a distribution, and each transition is associated with the probability of the admissable nucleotide.



Dai N., et al. arXiv preprint arXiv:2401.00037v2 (2024) .

3. mRNA Design Problem

- Calculation of **EFE** for a **DFA** is similar for general RNA sequences, with the exception that the sum over all sequences is replaced by the sum over all transitions in the DFA.
- Again, the Nussinov model is used to calculate the Gibbs Free Energy of a secondary structure.
- With a valid probabilistic DFA representation $\mathcal{S}$ and a differentiable loss function **EFE**, the authors apply projected gradient descent to optimize $\mathcal{S}$.
- Projected gradient descent is necessary to ensure that $p_{iA} + p_{iC} + p_{iG} + p_{iU} = 1$ for $p_i > 0$.
  - First, gradient descent $S'_{t+1} = S_t - \eta \nabla \text{EFE}(S_t)$ is performed.
  - Second, the projection step is performed where N is the number of transitions in the DFA.

$$S_{t+1} = \text{argmin}_S \left\{ \|S - S'_{t+1}\|_2^2 \mid S > 0, S \times \mathbf{1}_4 = \mathbf{1}_N \right\}$$

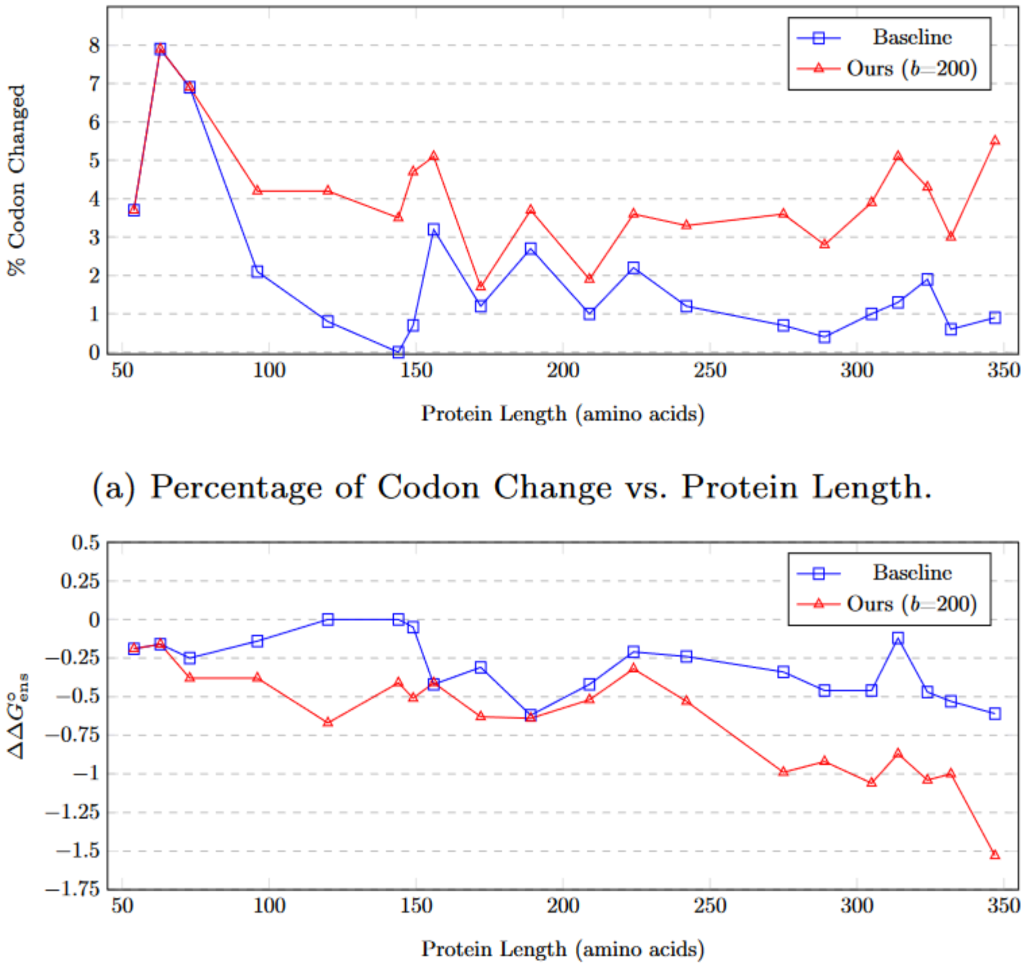
  - This process is repeated until convergence to a one-hot encoding of the optimal sequence.

4. Experimental Design and Results

4. Experimental Design

- The authors compared the EFE of their method to that of LinearDesign on a set of 20 random protein sequences from UniProt↗. The sequences are between 50 and 350 amino acids long.
- $\mathcal{S}$ is initialized with the minimal free energy sequence of the protein from **LinearDesign**. Taking $\mathcal{S}_0$ as the MFE solution leads to it being trapped, $(1-\epsilon)\mathcal{S}+\epsilon\mathcal{S}_{\text{rand}}$ is used with $\epsilon = 0.5$.
- 30 iterations of gradient descent are performed with a learning rate of 1 and beam size of 100.
- This is compared to a Random Walk (RW) baseline, where $\mathcal{S}$ is initialized with the same $\mathcal{S}_0$ as LinearDesign, and a random codon is selected at each iteration given a lower $-RT\log\mathcal{Z}$.
  - 100 iterations are performed.
- Evaluation is done by **LinearPartition**, with options `-V -b0`.

4. Results: Gradient Descent Outperforms Random Walk

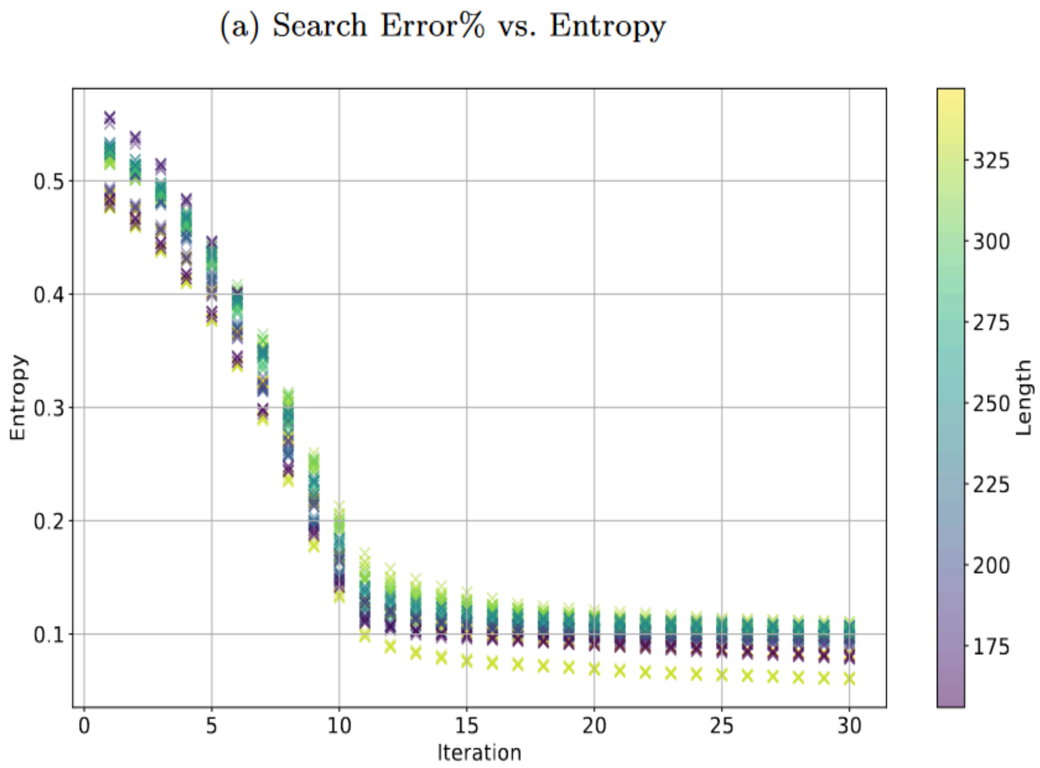


(a) Percentage of Codon Change vs. Protein Length.

- It is observed that the projected gradient descent method has a considerably wider search space than the random walk method.
- Furthermore the decrease in EFE is greater for the gradient descent method than for the random walk method.

Dai N., et al. arXiv preprint arXiv:2401.00037v2 (2024) .

4. Results: Beam Search Error Correlates with Entropy

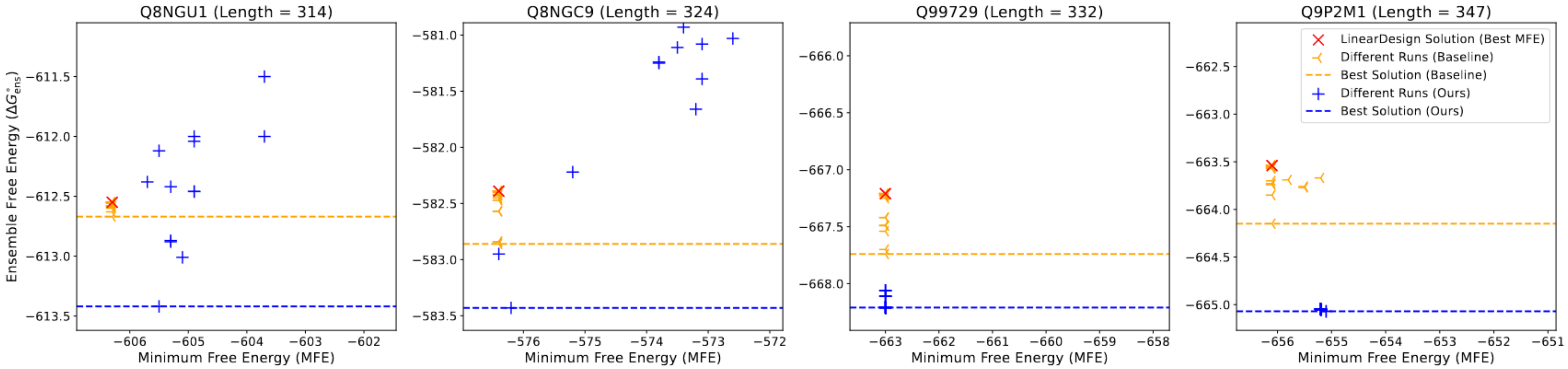


Dai N., et al. arXiv preprint arXiv:2401.00037v2 (2024) .

- With the classic Shannon entropy, the authors find that the level of entropy decreases as S converges.
- The error from beam search is measured by comparing the results with $b = 100$ and $b = \infty$.
- The authors find that the error from beam search is correlated with the level of entropy.

4. Results: Gradient Descent for Longer Sequences

- The authors find that the gradient descent may converge to a suboptimal solution for longer sequences.
- Even for longer sequences, the decrease in EFE is small
  - absolute difference of $< 1\text{kcal/mol}$
  - relative difference of $< 0.16\%$.



Dai N., et al. arXiv preprint arXiv:2401.00037v2 (2024) .

5. Summary and Discussion

5. Summary

- The authors propose a novel method for mRNA design that translates the problem from one of discrete optimization to one of continuous optimization.
- The authors define the Expected Partition Function $\overline{\mathcal{Z}}$ and apply gradient descent to optimize $\mathcal{S}$, a probability distribution over sequences either in the form of a $L \times 4$ matrix or a probabilistic DFA.
- The authors show that their method outperforms LinearDesign in terms of EFE, and that the error from beam search is correlated with the level of entropy.

5. Discussion

- Would an implementation of the Turner model be needed or feasible for practical applications?
    - A similar paper implementing the Turner model failed for sequences longer than 50 nucleotides due to memory constraints.
- Is a decrease of $< 1\text{kcal/mol}$ in the Energy Free Ensemble significant?
- Is the random walk baseline fair?
- Is the error from beam search a significant issue?
- How low should the entropy be before convergence?
- Is dual optimization possible?
    - It is trivial to define an expected CAI based on the geometric mean over the probabilistic DFA.

Q&A