# Gradient-based Optimization for mRNA Sequence Design The ID3 (Input Data Differentiable Designer) Framework

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Paper

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## Introduction

### The mRNA Design Challenge

- Critical for COVID-19 vaccines (Pfizer/BioNTech, Moderna)
- Multiple objectives: accessibility, stability, translation efficiency
- Hard constraint: Preserve amino acid sequence

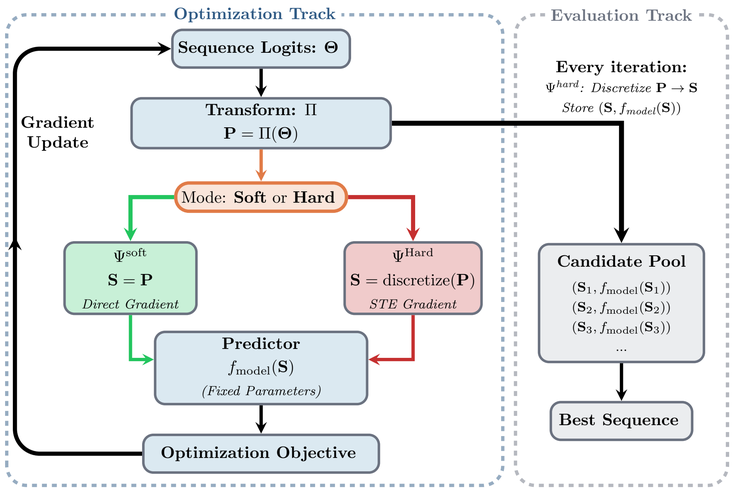
## The Problem

**The discrete-continuous gap prevents gradient-based optimization**

### Existing Approaches Fall Short:

1. **Discrete optimization** (GA, SA): Slow, no theoretical guarantees
2. **Straight-Through Estimator**: Lacks systematic constraint mechanisms
3. **No unified framework** for gradient optimization + biological constraints

## Core Innovation: Decoupled Architecture




**Key Insight:** Models accept probability distributions as input

### Core Objective:

$$\min_{\Theta} \mathcal{L} = \min_{\Theta} f_{\text{model}}(T(\Theta)), \quad T = \Psi \circ \Pi$$

### ID3 Pipeline:

$$\Theta \xrightarrow{\Pi} \mathbf{P} \xrightarrow{\Psi} \mathbf{S} \xrightarrow{f_{\text{model}}} \mathcal{L}, \quad \mathcal{L} \dashrightarrow \Theta \text{ via } \nabla$$

- $\Pi$: Parameter-to-Probability (Gumbel-Softmax)
- $\Psi$: Probability-to-Sequence (Soft/Hard modes)
- $\nabla$: Gradient-based updates

**Transformation Functions:**

*Parameter-to-Probability* ($\Pi$):

$$\mathbf{P}^{\text{det}} = \text{softmax}(\Theta/\sigma)$$
$$\mathbf{P}^{\text{sto}} = \text{softmax}((\Theta + g)/\sigma)$$

where $g$ is Gumbel noise, $\sigma$ is temperature parameter

*Probability-to-Sequence* ($\Psi$):

$$\psi^{\text{soft}}(\mathbf{P}) = \mathbf{P}$$
$$\psi^{\text{hard}}(\mathbf{P}) = \text{discretize}(\mathbf{P})$$

where discretize uses Straight-Through Estimator (STE)

**Four Operational Modes:**

| Mode | Parameter | Output |
|---|---|---|
| Det.Soft | Deterministic | Continuous |
| Det.Hard | Deterministic | Discrete |
| Sto.Soft | Stochastic | Continuous |
| Sto.Hard | Stochastic | Discrete |

## Three Constraint Mechanisms

**Challenge:** Optimize RNA while preserving amino acids

### 1. Codon Profile Constraint

*Parameter Domain:*

$$\Theta_{\text{codon}} = \{\theta_{j,c} : c \in \mathcal{C}(y_j)\}$$

*Probability Computation:*

$$\mathbf{P}_{\text{codon}} = \text{softmax}(\theta_{j,c}/\sigma)_{c \in \mathcal{C}(y_j)}$$

- Mathematically impossible to violate constraints
- 40-60% reduction: $\sum_{j=1}^{M} |\mathcal{C}(y_j)|$ vs $L \times 4$

### 2. Amino Matching Softmax

*Parameter Domain:*

$$\Theta \in \mathbb{R}^{L \times 4} \quad \text{(full RNA space)}$$

where $\Theta^{(j)} \in \mathbb{R}^{3 \times 4}$ for $j$-th amino acid's codon

*Probability Computation:*

$$\mathbf{P}_{\text{codon},c} = \text{softmax}(\langle \Theta^{(j)}, \mathcal{E}[c] \rangle / \sigma)_{c \in \mathcal{C}(y_j)}$$

- $\mathcal{E}[c] \in \{0,1\}^{3 \times 4}$: One-hot encoding of codon $c$
- $\langle \Theta^{(j)}, \mathcal{E}[c] \rangle$: Inner product similarity
- Only valid codons get non-zero probabilities

### 3. Lagrangian Multiplier

*Parameter Domain:*

$$\Theta \in \mathbb{R}^{L \times 4}, \quad \mathbf{P} \in [0,1]^{L \times 4}$$

*Total Loss with Penalty:*

$$\mathcal{L}_{\text{total}} = f_{\text{model}}(T(\Theta)) + \lambda \cdot \mathcal{C}(\mathbf{P})$$

*Constraint Violation Measure:*

$$\mathcal{C}(\mathbf{P}) = \frac{1}{M} \sum_{j=1}^{M} \min_{c \in \mathcal{C}(y_j)} \|\mathbf{P}^{(j)} - \mathcal{E}[c]\|^2$$

L2 distance to closest valid codon

*Adaptive Multiplier Update:*

$$\lambda^{(t+1)} = \max(0, \lambda^{(t)} + \eta_t \cdot g^{(t)}), \quad \eta_t = \eta_0/\sqrt{t+1}$$

Subgradient method with decaying step size

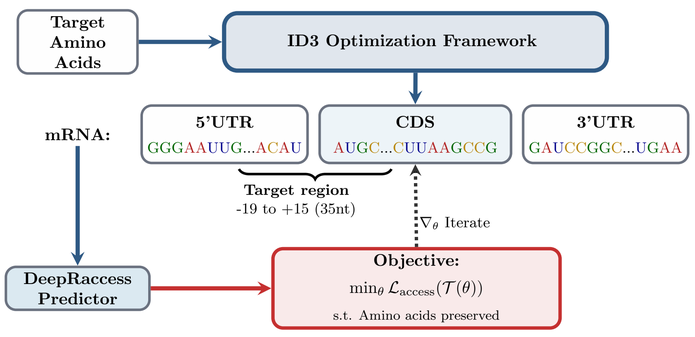
**4 Modes × 3 Mechanisms = 12 Variants**

## Applications

### 1. Accessibility Optimization with DeepRaccess[1]

*Loss Function:*

$$\mathcal{L} = f_{\text{access}}(T(\Theta))_{\text{ATG}-19:+15}$$
$$\text{s.t.} \quad \text{AA}(T^{\text{hard}}(\Theta)) = \mathbf{y}$$




*Goal: Minimize RNA accessibility in translation initiation region*

[1]DeepRaccess predictor: Hara et al., Frontiers in Bioinformatics, 3:1275787, 2023

### 2. CAI Co-Optimization

*CAI-aware Discretization:*

$$\Psi^{\text{hard}}_{\text{CAI}}(\mathbf{P}_{\text{codon}}) = \arg\max_{S} P(S|\mathbf{P}_{\text{codon}})$$
$$\text{s.t.} \quad \text{CAI}(S) \geq \text{threshold}$$

*Implementation:*

$$\mathbf{P}(\gamma) = \gamma \cdot \mathbf{w}_{\text{organism}} + (1-\gamma) \cdot \mathbf{P}_{\text{codon}}$$

Binary search finds optimal $\gamma \in [0,1]$ satisfying CAI threshold

*Alternative: Multi-objective Joint Loss*

$$\mathcal{L}_{\text{total}} = \mathcal{L}_{\text{access}} + \lambda_{\text{CAI}} \cdot \mathcal{L}_{\text{CAI}}$$

Gradient-based, flexible, works for both soft and hard modes

## Theoretical Guarantees

**First convergence proof for sequence optimization:** Gradient descent converges to stationary points under Lipschitz continuity, covering all 12 variants. See supplementary materials for detailed proofs.

## Experimental Setup

**Experiments:**

- 12 variants × 12 proteins
- 20 independent runs
- 1000 evaluations/run

**Baselines:**

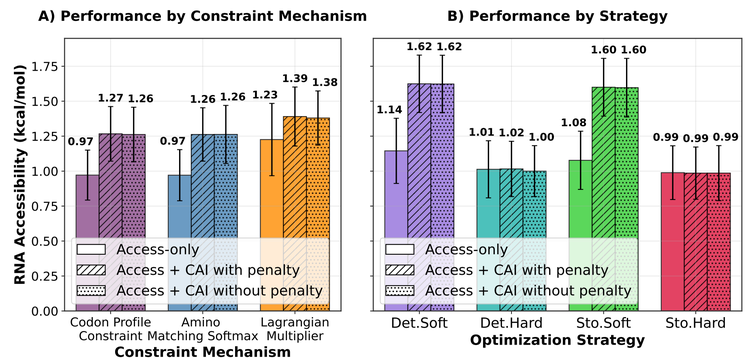
- Genetic Algorithm (GA)
- Simulated Annealing (SA)
- C10: Exhaustive (1.36M)

**Dataset (12 proteins, 64-1273 AA)**

| ID | Protein | AA |
|---|---|---|
| O15263 | Defensin beta 4A | 64 |
| P99999 | Cyt c (H.s.) | 105 |
| P00004 | Cyt c (E.c.) | 105 |
| P01308 | Insulin | 110 |
| P01825 | Immuno. HC | 116 |
| P31417 | FABP2 | 132 |
| P61626 | Lysozyme C | 148 |
| P42212 | GFP | 238 |
| P04637 | p53 | 393 |
| P0DTC9 | SARS-CoV-2 N | 419 |
| P0CG48 | Polyubiquitin-C | 685 |
| P0DTC2 | SARS-CoV-2 Spike | 1273 |

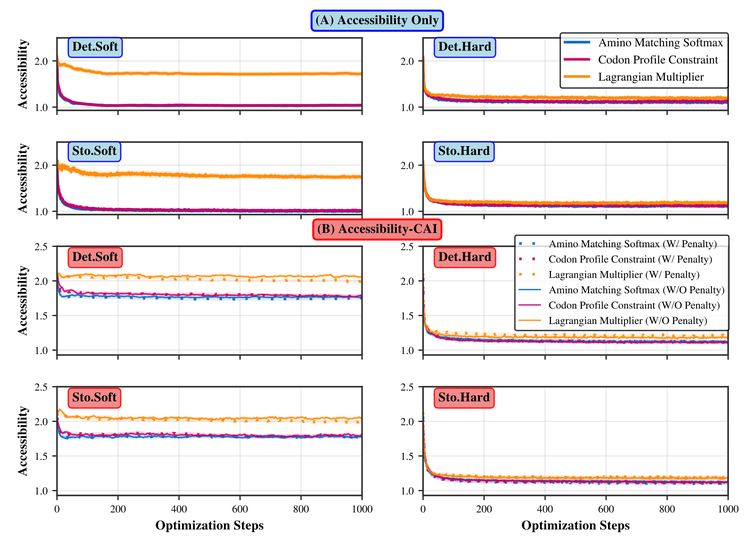
## Mechanism & Strategy Analysis

### Constraint Mechanism Comparison




Codon Profile and Amino Matching achieve similar performance

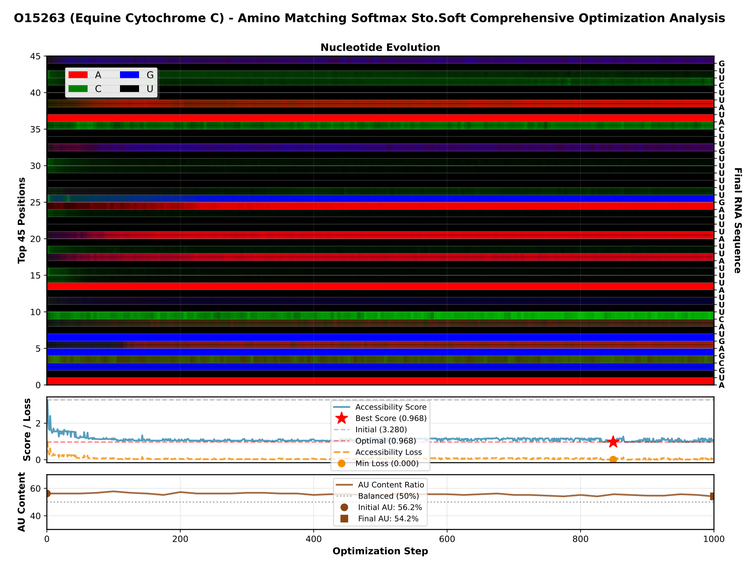
### Convergence by Strategy




Soft and hard comparable for single objective; hard superior for multi-objective

## Case Study: O15263 (Defensin beta 4A)

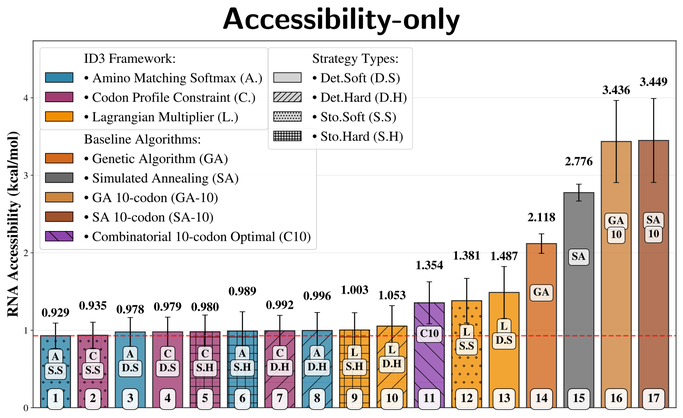
### Optimization Trajectory using Amino.Sto.Soft:




Rapid convergence: 3.28 → 0.97 kcal/mol with systematic AU balancing

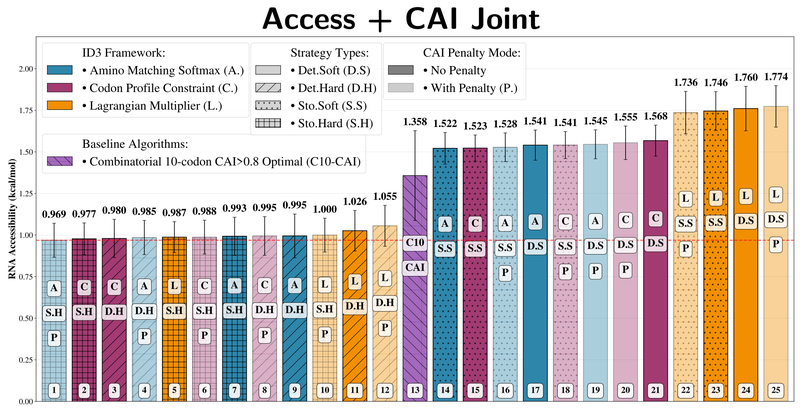
## Performance Comparison & Conclusion

### Accessibility-only




**10/12 variants outperform all baselines**

### Access + CAI Joint




**Hard constraints excel in multi-objective optimization**

### Conclusion

**Key Contributions:**

- Decoupled optimization-evaluation architecture
- Three systematic constraint mechanisms
- First convergence proof for sequence optimization
- 12 systematic variants covering optimization landscape
- Superior performance across diverse proteins

**Impact:**

- Principled foundation for gradient-based discrete sequence optimization
- Applications in mRNA vaccines and therapeutics
- Extensible to DNA and protein design

**Reference:** Li Hongmin, Goro Terai, Takumi Otagaki, Kiyoshi Asai. "Gradient-based Optimization for mRNA Sequence Design." *bioRxiv* 2025.10.22.683691

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