

# Construction of Free Energy Model

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## Abstract

Our model aims to investigate the off-target problem in gene editing by the CRISPR-Cas system, therefore finding efficient ways to enhance the reliability of gene editing. The foundations of this model are mostly simple probability theory and dynamic deduction, which make our model both convincing and pellucid.

## References

- [1] Misha Klein et al. "Hybridization kinetics explains CRISPR-Cas off-targeting rules". In: *Cell reports* 22.6 (2018), pp. 1413–1423.

## 1 Introduction

Currently, people have constructed a similar model as illustrated in the following figure (Figure 1). There are four common rules when Cas nuclease cleaves the DNA [KLEIN20181413].

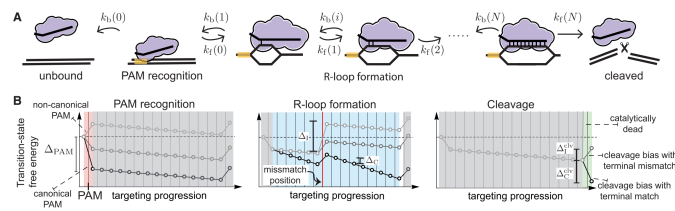


Figure 1: schematic diagram

- (1) Seed region: single mismatch(es) within a PAM proximal seed region can completely disrupt interference
- (2) Mismatch spread: when mismatches are outside the seed region, off-targets with spread out mismatches are targeted most strongly
- (3) Differential binding versus differential cleavage: binding is more tolerant of mismatches than cleavage
- (4) Specificity-efficiency decoupling: weakened protein-DNA interactions can improve target selectivity while still maintaining efficiency

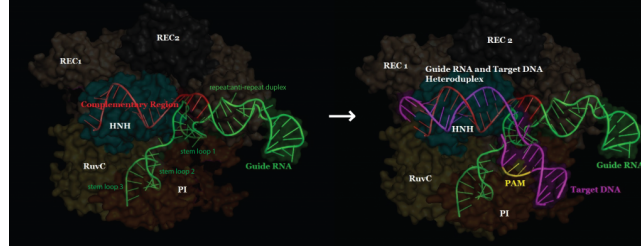


Figure 2

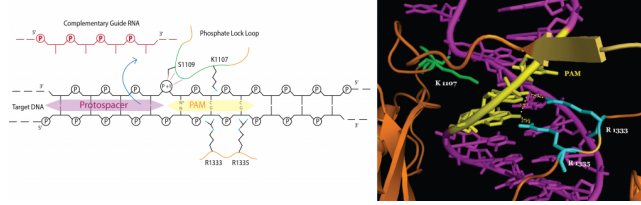


Figure 3

Based on these four rules, probability theory is applied in to explain it. As we know, there are always only two results in an experiment, which are successful cleavage and unsuccessful cleavage. In math view, it can be one-hot encoded, and they are corresponding to 1 and 0.

To solve this problem, one choice is to consider it as a cluster problem; however, it is easier to find a continuous quantitative function rather than to find a suitable cluster distance function. So naturally, finding an approximate probability distribution is a good choice.

In many target design toolkits, they use a score function with several parameters which can generate a score to evaluate whether the target is good or bad. Here we consider the score function has the similar ability to probability, which is a description of "better" or "worse" while can't affirm whether successful cleavage will appear. For our case, our goal is to find a function indicating which target is BETTER.

Considering the difference between model prediction and experimental data, our model consists of two aspects, which are kinetic inference and an updating module.

## 2 Models

### 2.1 Kinetic model

Figure 2 shows that the whole binding-cleavage process begins with the binding between PAM and protein. Therefore, it corresponds to rule 1 mentioned before. And as the reaction proceeds, every step of it is reversible, and its irreversibility mainly depends on the binding energy of two DNA bases.

The boundary probability  $P_{clw;N}$ , representing the probability to cleave starting with a full R-loop and without reducing the R-loop's length, is given by a simple

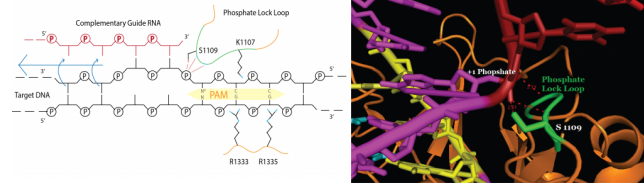


Figure 4

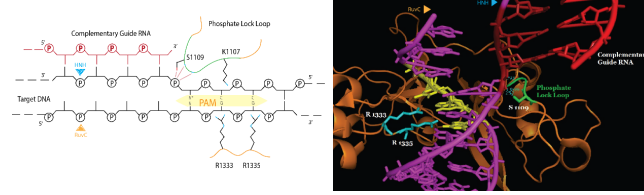


Figure 5

splitting probability:

$$P_{clv,N} = \frac{k_f(N)}{k_f(N) + k_b(N)} = \frac{1}{1 + \gamma_N} \quad \gamma_N = \frac{k_b(N)}{k_f(N)}$$

$$P_{clv} \equiv P_{clv,0} = \frac{1}{1 + \sum_{k=1}^N \prod_{i=1}^n \gamma_i}$$

free-energy:  $F_i$ ; the transition state energy:  $T_i$ , from  $i$  to  $i + 1$ , we can get the relationship between reaction constant and free energy:

$$k_f(i) = k_0 \exp(-(T_i - F_i)), k_b(i) = k_0 \exp(-(-T_{i-1} - F_i))$$

$$\Rightarrow \gamma_i = \exp(-\Delta_i), \Delta_i = T_i - T_{i-1}$$

$$\Rightarrow P_{clv} = \frac{1}{1 + \sum_{k=1}^N \prod_{i=1}^n \gamma_i} = \frac{1}{1 + \sum_{k=1}^N \prod_{i=1}^n \exp(-\Delta_i)} = \frac{1}{1 + \sum_{k=1}^N \exp(-\sum_{k=1}^n \Delta_i)}$$

define:

$$\Delta T_n = \sum_{k=1}^n \Delta_i$$

$$P_{clv} = \frac{1}{1 + \sum_{k=1}^N \exp(-\Delta T_n)}$$

From the above it is clear that the cleavage probability depends only on the transition state energies, and not on the free energies of the metastable states. If we assume there to be one dominant minimal bias, say for  $n = n$ , then this can be approximated as:

$$P_{clv} \approx \frac{1}{1 + \exp(-\Delta T_n)}$$

As figure shows, we analyse each process energy change:

- { for the PAM state ( $i = 0$ ) we have  $\Delta_0 = PAM$
- { for a partial R-loop we have  $\Delta_i = \Delta_C$  and  $\Delta_i = \Delta_I$  if mismatched

$$\Delta T_n = \Delta_{PAM} + n_C(n) \Delta_C (n n_C(n)) \Delta_I \delta_{n,N} \Delta_{clv}; n = 0 \dots N$$

where  $\delta_{n,N}$  represents the *Kronecker* delta:  $\delta_{n,N} = \begin{cases} 1, n = N; \\ 0, n \neq N. \end{cases}$

For PAM independent systems (such as cas13), we instead use:

$$\Delta T_n = n_C(n) \Delta_C(n n_C(n)) \Delta_I \delta_{n,N} \Delta_{clv}; n = 0 \dots N$$

To sum up, the cleavage possibility mainly rely on the free energy change, and PAM appears as a large energy decline.

So the kenetic module set up a form to regression the relationship between cleavage and the numbers of nucleotide matches and mismatches. Considering this problem more carefully, the cleavage possibility become equal to analysis energy change and simply we know the binding energy of A/T and C/G is different due to the different hydrogen bond between them. However, in appearing kinetic model, research tend to describe them in a rough definition as "matched base pairs" and the energy incline in C/G is approximately 1.5 folds as A/T. Similarly, the mismatch has more difference because the size of nucleotides is various. Hence, the combination of mismatched base pair was classified by group volume, i.e. two pyrimidine (such as C/T, "L"), pyrimidine and purine (such as C/A, "M"), two purine (such as G/T, "S"). Hence, our possibility can be calculated using following formation.

$$P_{clv} = \frac{1}{1 + e^{-n_1 \Delta_{A/T} - n_2 \Delta_{C/G} + n_3 \Delta_L + n_4 \Delta_M + n_5 \Delta_S}}$$

## 2.2 Optimization model

It is common sense that experiment result are facts but theoretical results are only conjectures. In the model 1, we can get an output, which means the cleavage possibility. The parameter we choose only aim to make results has discrimination, while it's not quantitative. And in a cleavage experiment, we only have two results, successful and unsuccessful. To make our prediction possibility more approximate to experiment, we regard this as a regression problem.

Here, the method we choose is stochastic gradient descent (SGD) and choose cross entropy. And their principle can be concluded as following.

$$\theta = \theta - \eta \nabla_{\theta} J(x^{(i)}, y^{(i)}, \theta)$$

$$loss = \sum_i y_i \ln y_i$$

where  $\theta$  means the parameters array and J means the loss function,

Considering the difference in gradient calculation, we use difference to substitute differential aim to accelerate operating speed.

$$\frac{dy}{dx} \approx \frac{\Delta y}{\Delta x} = \frac{y(x + \delta x) - y(x)}{\delta x}$$

By using this simple method, our model can be more vibrant, updating using newest data and becoming more reliable.

## 2.3 Pre-selector

It's obviously that the algorithm is too complex to applying in slide in a huge DNA array. To solve this problem, we use a pre-selector to get some candidates and use previous model to compare them so that we could get a greatest target.

And here this pre-selector structure is very simple. Considering use this map to reflect the similarity between target and full DNA.

$$f(a_{\text{target}} - a_{\text{full}}) = \mathbf{x} = (x_i)$$

$$x_i = \begin{cases} 1, & \text{matched} \\ 0, & \text{mismatched} \end{cases}$$

Here, we use PAM as an input and collect the array which contain the same beginning code as PAM.

### 3 Result

Here, we set this parameters as default values and observe its performance. As the following figure shows, the energy is always declined or has some turning point and is always negative. Such as the red line, it have a peak due to a mismatch here, and in our model we find that it doesn't make the energy positive. That means that in this reaction process there are some force somewhat like momentum pushing it proceed and cross the energy peak. Corresponding to the other figure's two special location(a ad b), only in these point their energy are all negative (because we want to see the idea target series, only the locaitons which corresponds to negative energy are collected).

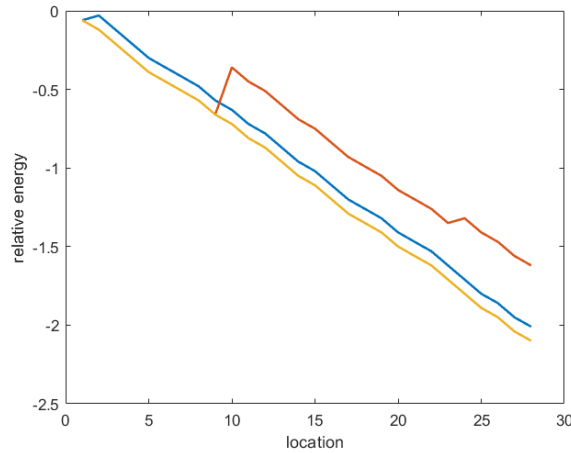


Figure 6: energy change

Besides the default parameters, we hope our model can hit more ture data. So if we get the experiment datas, we can use model 2.2 to get greater parameters.(@@no experiment data) cited

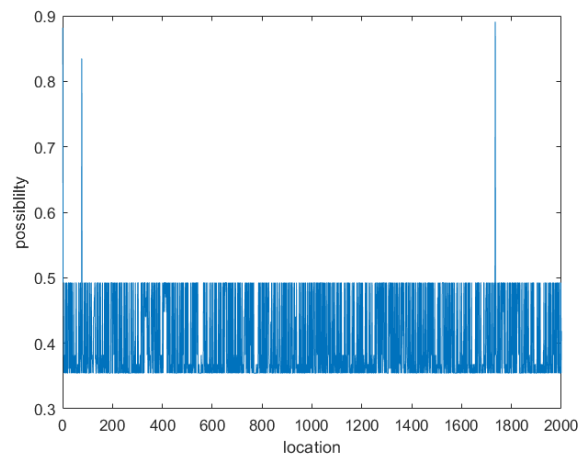


Figure 7: the possibility of target binding to nucleotide array in different location