Physical limits of CRISPR-SpCas9 specificity

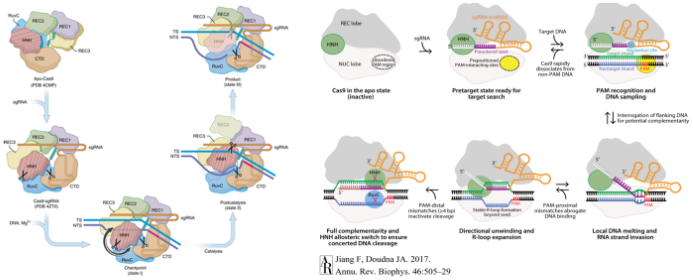
Sam Padula[1], Chris Nam[2], Jeremy Gunawardena[2]

1: Wofford College, Papadopolous Fellowship | 2: Harvard Medical School

Big Picture

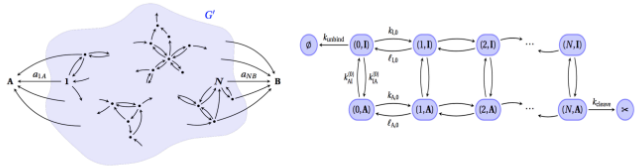
This project is a means to exploring the idea that biochemical information processing systems that are not coupled to external energy sources have bounded efficacies. The series of steps preceding the cleavage of dsDNA by SpCas9 is believed to not be coupled to any external energy sources, which raises the question of whether there is a physical limit to the specificity of SpCas9. To answer this question, we are using a graph-based probabilistic framework to model the series of steps preceding the cleavage of dsDNA by SpCas9.

Background



Jiang F, Doudna JA. 2017. Annu. Rev. Biophys. 46:505–29

Methods



$$\Pr(X(t) = B \text{ for some } t > 0 \mid X(0) = 1) = \left(\sum_{F \in \Phi_{\{A,1\}\to\{A,B\}}(G)} w(F)\right) \Big/ \left(\sum_{F \in \Phi_{\to\{A,B\}}(G)} w(F)\right)$$

$$= \left(a_{NB} \sum_{F \in \Theta_{\to N}(G')} w(F)\right) \Big/ \left(a_{1A} \sum_{F \in \Theta_{\to 1}(G')} w(F) + a_{NB} \sum_{F \in \Theta_{\to N}(G')} w(F) + a_{1A} a_{NB} \sum_{F \in \Phi_{\to\{1,N\}}(G')} w(F)\right)$$

$$= \left(1 + \left(a_{1A} \sum_{F \in \Theta_{\to 1}(G')} w(F) + a_{1A} a_{NB} \sum_{F \in \Phi_{\to\{1,N\}}(G')} w(F)\right) \Big/ \left(a_{NB} \sum_{F \in \Theta_{\to N}(G')} w(F)\right)\right)^{-1}. \quad (4.1)$$

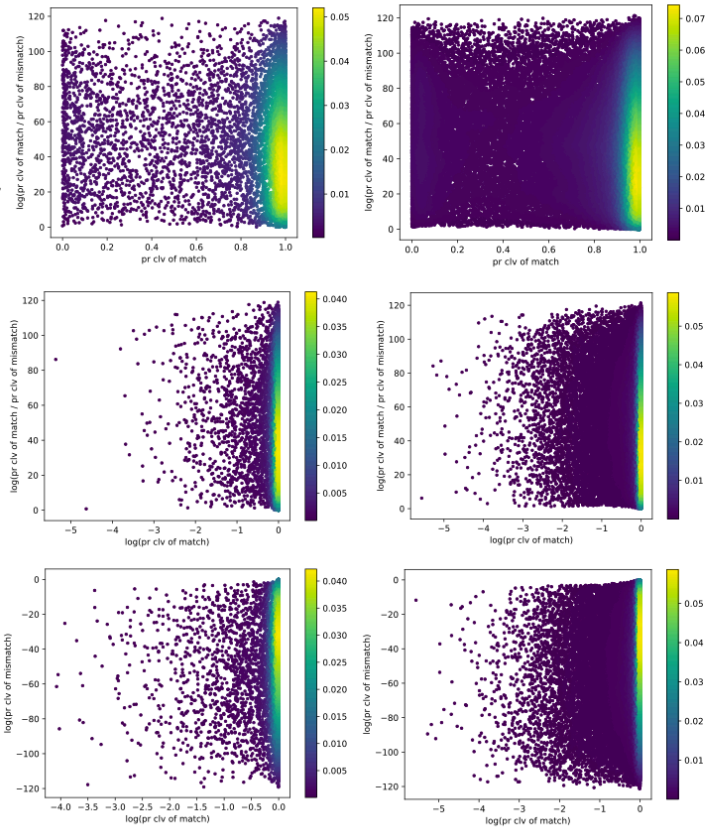
Methods continued

Six, simple parameters for preliminary model 1:

1. rate of hybridization (hyb) for a match > rate of dehybridization (dehyb) for a match
2. rate of dehyb. for a mismatch > rate of hyb. for a mismatch
3. rate of hyb. for a match > rate of hyb. for a mismatch
4. rate of dehyb. for a mismatch > rate of dehyb. for a match
5. rate of inactivation of HNH domain
6. rate of activation of HNH domain

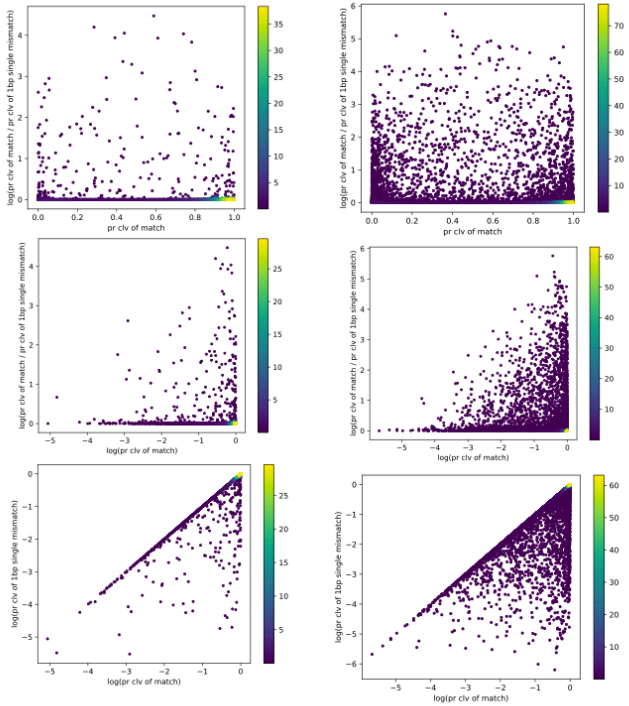
Results – 20bp mismatch

10k points range(0.001,1000) 100k points



Results – 1bp PAM-distal mismatch

10k points range(0.001,1000) 100k points



Conclusions and next steps

Because of our initial assumptions, you'd expect for the probability of cleavage of a match to be greater than the probability of cleavage of a mismatch. So, log (pr clv of match / pr clv of mismatch) you'd expect to be greater than 0. Also, you'd expect for the probability of cleavage of a 20bp mismatch sequence to be several orders of magnitude lower than the probability of cleavage of a 1bp PAM-distal mismatch. These results show all of these. We need to continue to explore the data generated in this preliminary model. Later, we plan to impose more conditions, for example, by incorporating differences in the rates of activation and inactivation of the HNH domain into the model.

References

- Zhu et al. 2019 – Cryo-EM structures reveal coordinated domain motions that govern DNA cleavage by Cas9 – Nature Structural & Molecular Biology
- Jiang & Doudna 2017 – CRISPR-Cas9 Structures and Mechanisms – Annual Rev. Biophys