Stochastic Model and Optimization of SELEX

Yue Wang

Department of Computational Medicine
University of California, Los Angeles

Joint work with Bhaven A. Mistry and Tom Chou

yuew@g.ucla.edu

Outline

- Introduce SELEX: a process to select aptamers.
- Review the traditional deterministic model.
- Build a stochastic model for SELEX and analyze its properties.
- Search for the optimal protocol of SELEX.

Section I: Introduction

- Aptamers are short, single-stranded DNA or RNA molecules that bind to a specific target.
- Targets can be heavy metal ions, proteins, or even whole cells.
- Certain aptamers (linked with fluorescent tracers) can bind selectively to biomarkers on the cancer cells, but not to healthy cells. This test can identify cancer cells in a tissue sample.
- Besides testing, aptamers can also be used in treatment. Therefore, aptamers are also called chemical antibodies.

Section I: Introduction

- It is difficult to design and synthesize the best aptamer for a target directly.
- In general, we start with enough targets and a large library of randomly generated aptamers, and aptamers have different affinities to the target.
- How to select the best aptamers (with the highest affinities to the targets) in an easy way?

Section I: Introduction

- Systematic Evolution of Ligands by EXponential enrichment (SELEX): a convenient method to select the best aptamers.
- Aptamers with higher affinities to the targets are more likely to bind to the targets. We can use the targets to pick out such aptamers.
- It is similar to a population evolution process.

Aptamers and targets can bind and unbind reversibly.

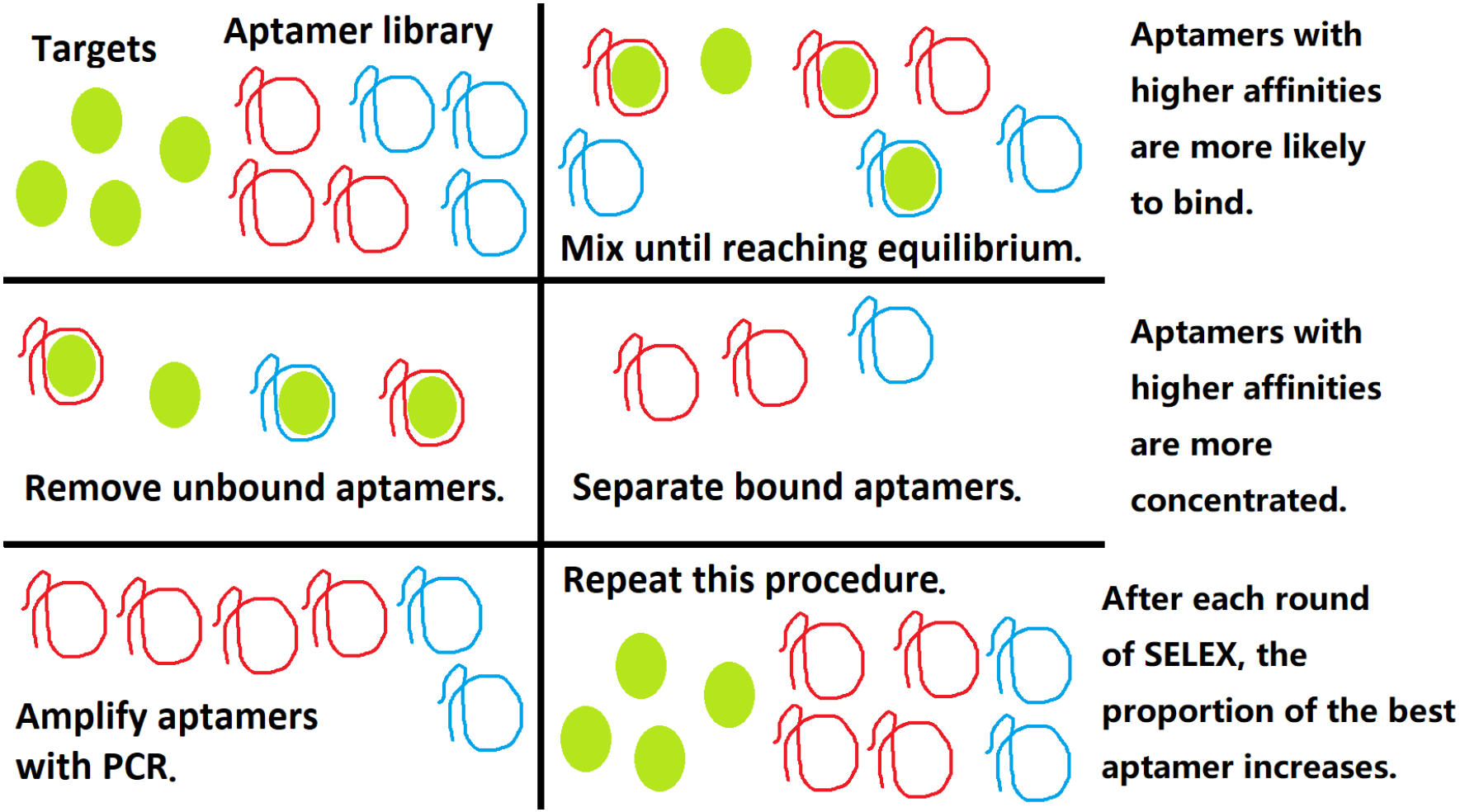


Figure: Protocol of SELEX

- We have enough targets, and the aptamers can be amplified by PCR. When starting one round of SELEX, we can control the quantity of targets and the quantity of aptamers, but the proportions of different aptamer types cannot be controlled.
- We obtain an optimization problem: maximize the proportion of the best aptamer (with the highest affinity) after this round of SELEX.
- A bad choice: add too many targets, so that almost all aptamers are bound. No selection is made, and the proportion of the best aptamer is invariant.

Section I: Introduction

- We need a mathematical model to study the optimization of SELEX.
- To simplify the discussion, we combine aptamers with different affinities into two types: strong type $\mathtt{A}_1$, weak type $\mathtt{A}_2$. The association constants (affinities) satisfy $K_1 > K_2$.

Section II: Deterministic Model

- A traditional deterministic approach uses the law of mass action, which is valid when the number of molecules is sufficiently large.
- Notations: $[S]$: total concentration of targets; $[A_i]$: total concentration of aptamer type $\mathtt{A}_i$; $[a_i]$: concentration of aptamers $\mathtt{A}_i$ that are bound to targets at equilibrium.
- At stationary, for each $i = 1, 2$ and the reaction $\mathtt{S} + \mathtt{A}_i \rightleftharpoons \mathtt{SA}_i$, we have:

$$([S] - [a_1] - [a_2])\,([A_i] - [a_i])\,K_i = [a_i].$$

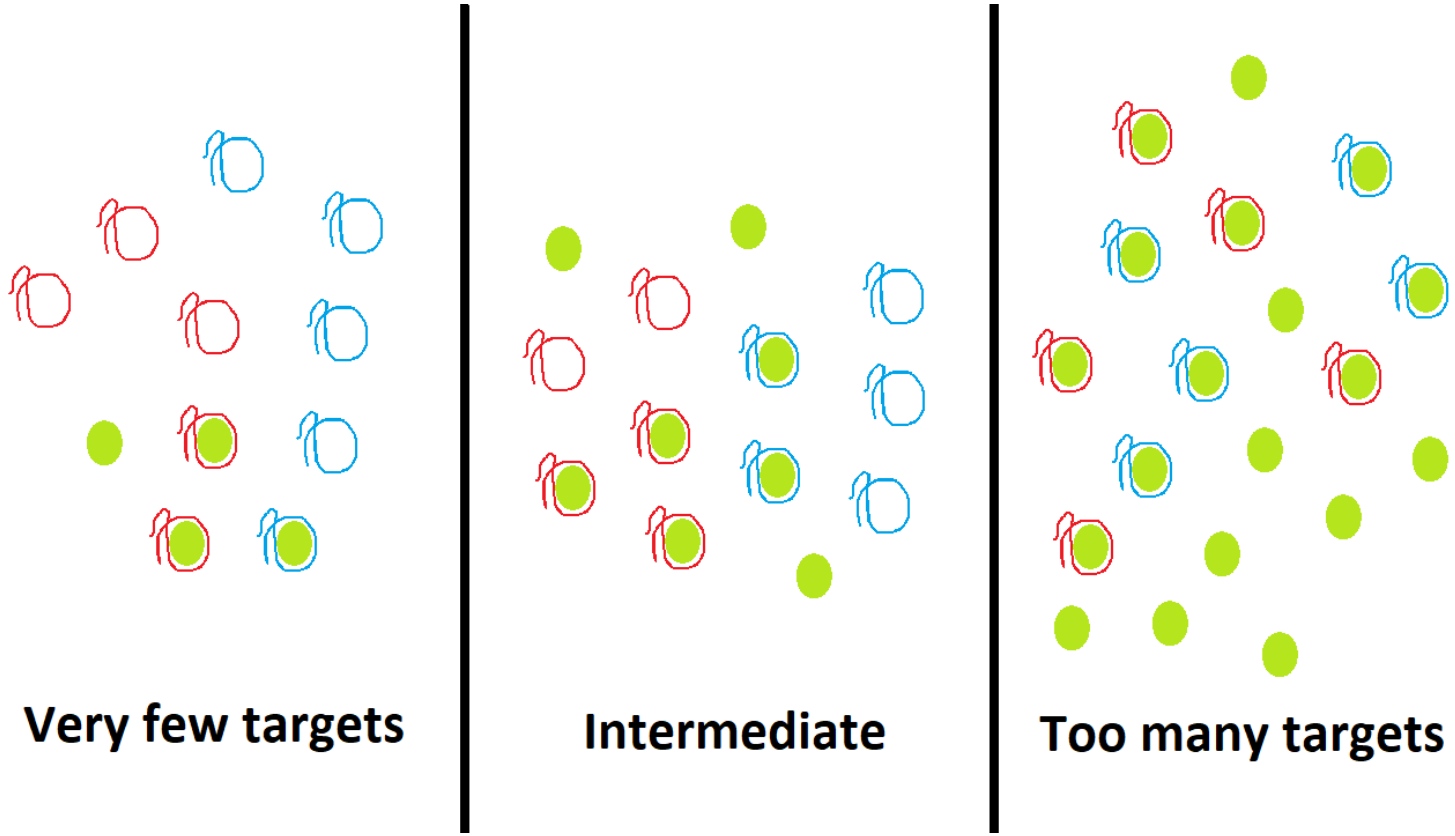
unbound target | unbound aptamer | bound aptamer

- Given $[S], [A_1], [A_2], K_1, K_2$, we can solve $[a_1], [a_2]$.

Section II: Deterministic Model

- For $\mathtt{A}_1$, the stronger aptamer, the goal is to maximize its proportion in bound aptamers: $[a_1]/([a_1]+[a_2])$.
- We can set different values of target concentration $[S]$ and aptamer concentration $[A_1]+[A_2]$, but the ratio $[A_1]/[A_2]$ is fixed.
- In this deterministic model, $[a_1]/([a_1]+[a_2])$ increases with $[A_1]+[A_2]$, and decreases with $[S]$.
- The optimal policy in the deterministic model: add as many aptamers as possible, and as few targets as possible.

Optimal policy: $[A_i] \gg [S]$.



$$\frac{[a_1]}{[a_1]+[a_2]} = \frac{[A_1]K_1}{[A_1]K_1+[A_2]K_2}$$

upper bound

$$\frac{[a_1]}{[a_1]+[a_2]} = \frac{[A_1]}{[A_1]+[A_2]}$$

lower bound

Section II: Deterministic Model

- The optimal policy in the deterministic model requires very large aptamer concentration $[A_1] + [A_2]$ and very small target concentration $[S]$.
- When $[S]$ is too small, randomness is inevitable, and the law of mass action does not hold.
- We need a stochastic model.
- We will show that something is different in this stochastic model.

Section III: Stochastic Model

- Notations: S: total number of targets; A_i: total number of $\mathbb{A}_i$ type aptamers; a_i: number of $\mathbb{A}_i$ aptamers that are bound to targets. $\bar{K}_i = K_i/V$: reaction coefficient, where V is the system volume.
- Consider a continuous-time Markov chain on 2-dimensional lattice $\mathbb{Z}^2$, where the states are the bound aptamer counts (a_1, a_2).
- The transition rates satisfy

$$\frac{r[(a_1,a_2)\to(a_1+1,a_2)]}{r[(a_1+1,a_2)\to(a_1,a_2)]} = \frac{(S-a_1-a_2)(A_1-a_1)}{a_1+1}\bar{K}_1.$$

$$\frac{r[(a_1,a_2)\to(a_1,a_2+1)]}{r[(a_1,a_2+1)\to(a_1,a_2)]} = \frac{(S-a_1-a_2)(A_2-a_2)}{a_2+1}\bar{K}_2.$$

- The stationary probability distribution satisfies

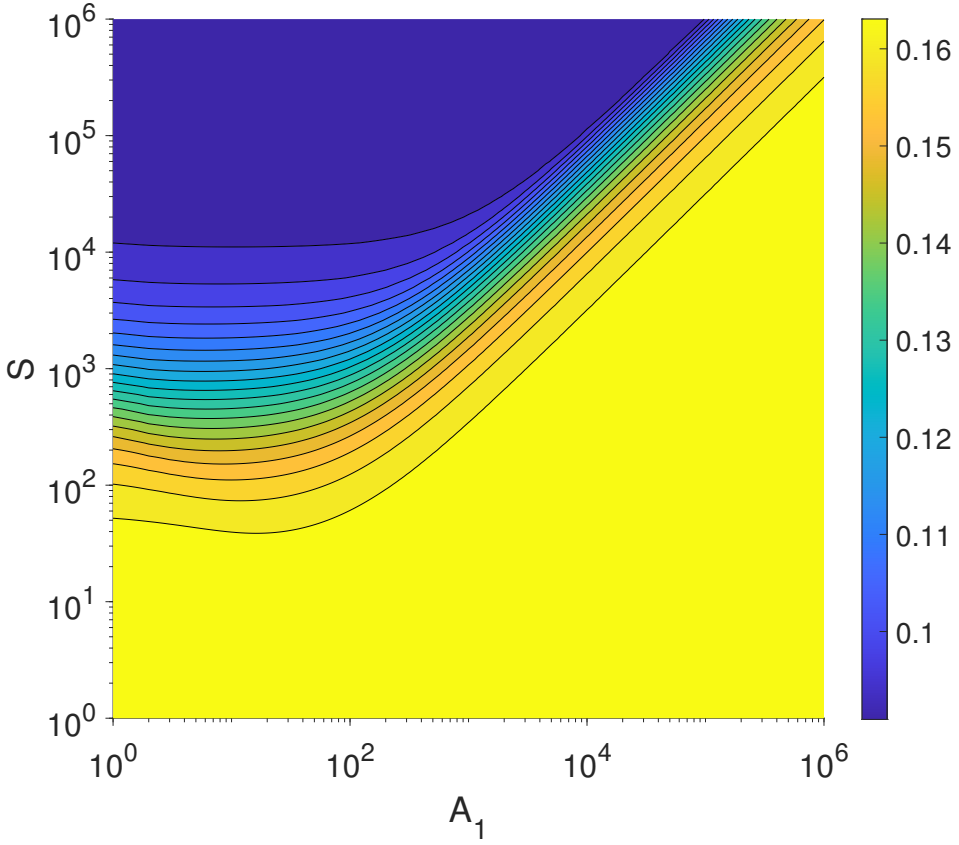
$$\mathbb{P}(a_1, a_2) = \mathbb{P}(0,0) \times \binom{S}{S - a_1 - a_2, a_1, a_2} \times \left[\binom{A_1}{a_1} \binom{A_2}{a_2} \right] \times [a_1! a_2!\,] \times \left[\bar{K}_1^{a_1} \bar{K}_2^{a_2} \right]$$

- Due to stochasticity, we need to consider the expected proportion of $\mathbb{A}_1$ and exclude the case that no aptamer is bound: $\mathbb{E}[a_1/(a_1 + a_2) \mid a_1 + a_2 > 0]$.

Section III: Stochastic Model

- In the deterministic model, $[a_1]/([a_1]+[a_2])$ increases with $[A_1]$ (fix $[A_1]/[A_2]$) and decreases with $[S]$.
- In the stochastic model, $\mathbb{E}[a_1/(a_1+a_2) \mid a_1+a_2>0]$ decreases with S, but does not always increase with A_1 (fix A_1/A_2).
- Set $S=10, \bar{K}_1=2, \bar{K}_2=1$.
  When $A_1=A_2=1$, $\mathbb{E}[a_1/(a_1+a_2) \mid a_1+a_2>0]=0.524$.
  When $A_1=A_2=2$, $\mathbb{E}[a_1/(a_1+a_2) \mid a_1+a_2>0]=0.521$.
- The problem is from the situation that $a_1=a_2=0$, which does not appear in the deterministic model.

Contour plot of the $\mathtt{A}_1$ proportion $\mathbb{E}[a_1/(a_1+a_2) \mid a_1+a_2>0]$ as a function of aptamer number A_1 (fix A_1/A_2) and target number S: it is monotonic with S, but not A_1.



In the stochastic model, we still have similar bounds for $\mathtt{A}_1$ proportion.

Theorem

$$\mathbb{E}[a_1/(a_1+a_2) \mid a_1+a_2>0] \leq A_1\bar{K}_1/(A_1\bar{K}_1+A_2\bar{K}_2).$$

Theorem

$$\mathbb{E}[a_1/(a_1+a_2) \mid a_1+a_2>0] \geq A_1/(A_1+A_2).$$

- Sketch of proof for the upper bound:
- Consider another system with $A_1\bar{K}_1/\bar{K}_2$ molecules of aptamer $\mathbb{A}'_1$ with reaction coefficient $\bar{K}_2$, A_2 molecules of aptamer $\mathbb{A}'_2$ with reaction coefficient $\bar{K}_2$, and S molecules of target.
- In this new system, two types of aptamers are the same. Due to symmetry,

$$\mathbb{E}[\frac{a'_1}{a'_1+a'_2} \mid a'_1+a'_2>0]$$
$$=\frac{A_1\bar{K}_1/\bar{K}_2}{A_1\bar{K}_1/\bar{K}_2+A_2}=\frac{A_1\bar{K}_1}{A_1\bar{K}_1+A_2\bar{K}_2}.$$

- We just need to prove $\mathbb{E}[a_1/(a_1+a_2) \mid a_1+a_2>0] \leq \mathbb{E}[a'_1/(a'_1+a'_2) \mid a'_1+a'_2>0]$.

- Sketch of proof for the lower bound:
- Consider another system with A_1 molecules of aptamer $\mathbb{A}_1''$ with reaction coefficient $\bar{K}_2$, A_2 molecules of aptamer $\mathbb{A}_2''$ with reaction coefficient $\bar{K}_2$, and S molecules of target.
- In this new system, two types of aptamers are the same. Due to symmetry,

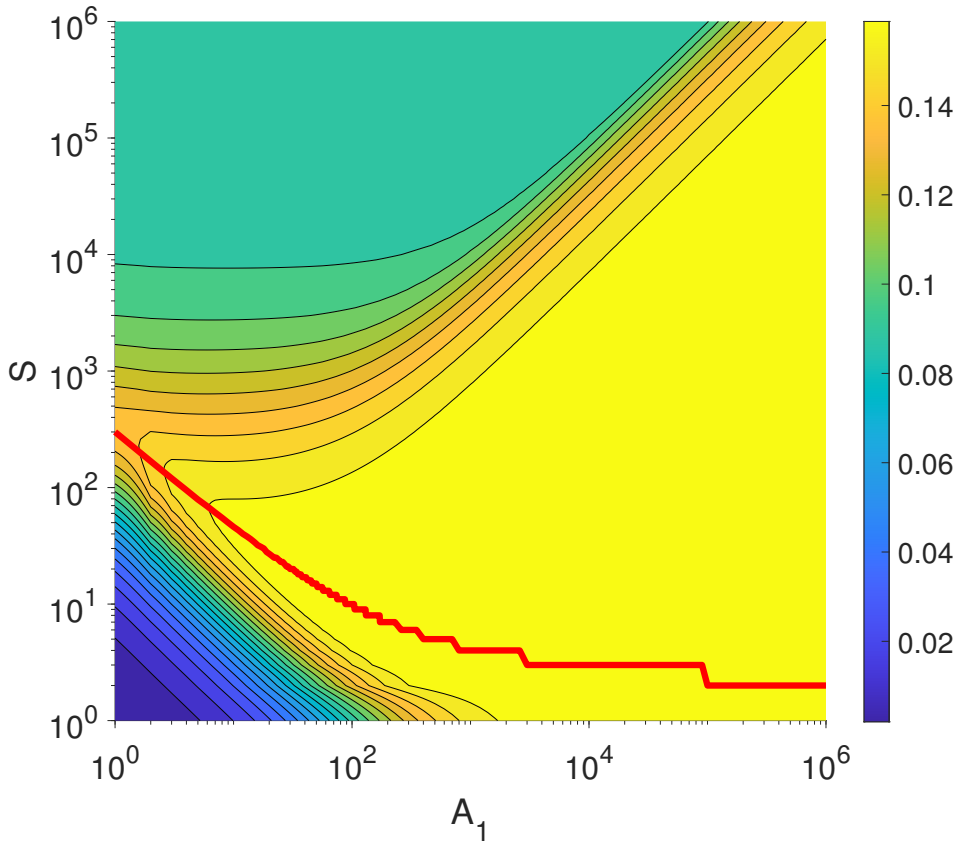
$$\mathbb{E}[\frac{a_1''}{a_1''+a_2''} \mid a_1''+a_2''>0] = \frac{A_1}{A_1+A_2}.$$

- We just need to prove $\mathbb{E}[a_1/(a_1+a_2) \mid a_1+a_2>0] \geq \mathbb{E}[a_1''/(a_1''+a_2'') \mid a_1''+a_2''>0]$.

Section III: Stochastic Model

- Studying $\mathbb{E}[a_1/(a_1+a_2) \mid a_1+a_2>0]$ is to show that the stochastic model has the same upper and lower bounds.
- In practice, we only want $\mathbb{A}_1$ aptamers. When $a_1=a_2=0$, we can stipulate that $a_1/(a_1+a_2)=0$.
- $\mathbb{E}[a_1/(a_1+a_2)] = \mathbb{E}[a_1/(a_1+a_2) \mid a_1+a_2>0] \times [1-\mathbb{P}(0,0)]$.

Section III: Stochastic Model



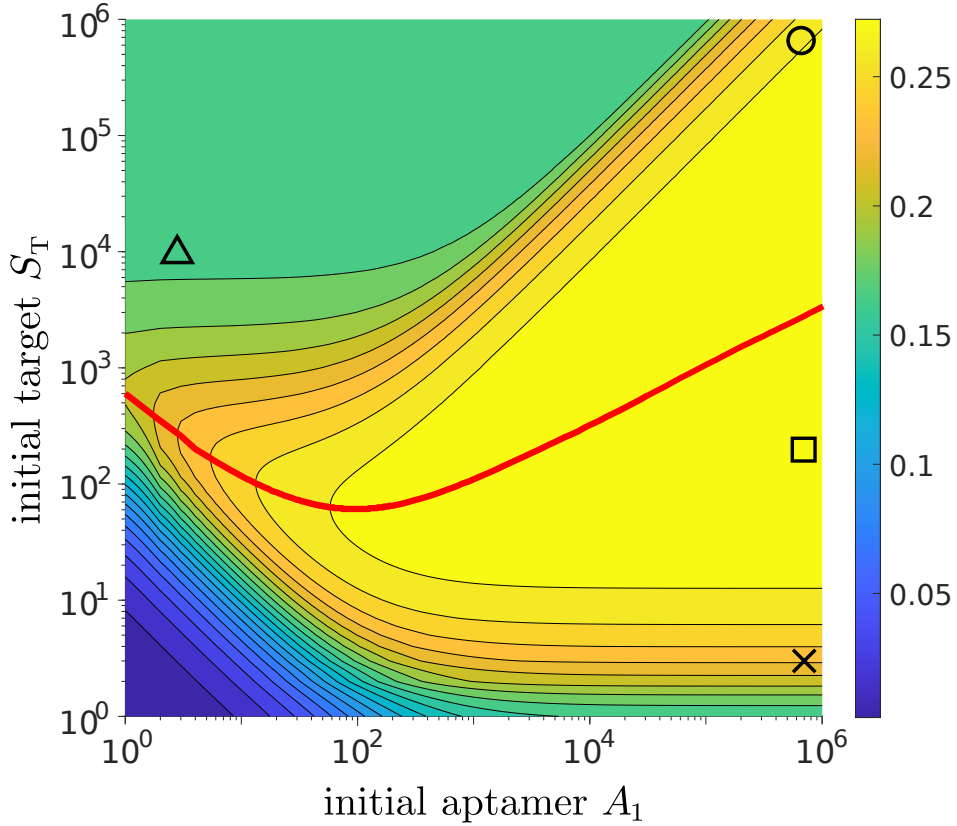
In the contour plot of $\mathbb{E}[a_1/(a_1 + a_2)]$, when the aptamer number A_1 (fix A_1/A_2) increases, the optimal target number S (red curve) decreases.

- Optimal policy in the stochastic model:
- When the aptamer number A_1 (and A_2) is not large, the target number S should not too small. Otherwise, $\mathbb{P}(a_1 = 0, a_2 = 0)$ might be large.
- What if we make A_1 (and A_2) sufficiently large, so that $\mathbb{P}(a_1 = 0, a_2 = 0) \approx 0$? Can we set $S = 1$ now?

- For one round of SELEX, $S = 1$ and very large A_1, A_2 can reach the upper bound:
  $\mathbb{E}[a_1/(a_1 + a_2)] \approx A_1\bar{K}_1/(A_1\bar{K}_1 + A_2\bar{K}_2)$.
- However, since there is only one target molecule, after one round of SELEX, only one aptamer type is left.
- After further rounds of SELEX, the expected $\mathbb{A}_1$ proportion does not increase.

Section III: Stochastic Model

- Contour plot of the $\mathbb{A}_1$ proportion $\mathbb{E}[a_1/(a_1 + a_2)]$ after two rounds of SELEX:



- For the first round, a policy with large A_1 and very small S does not perform well. The optimal target number S (red curve) first decreases and then increases with A_1.

Section III: Stochastic Model

- Theorem: Optimal policy for multiple rounds of SELEX in the stochastic model is $A_1, A_2 \gg S$ and $S \gg 1$.
- After N rounds of SELEX, this policy has $\mathbb{E}[a_1/(a_1+a_2)] \approx A_1\bar{K}_1^N/(A_1\bar{K}_1^N + A_2\bar{K}_2^N)$.
- Thus $1 - \mathbb{E}[a_1/(a_1+a_2)]$ converges to 0 exponentially fast with the rate $\approx \bar{K}_2/\bar{K}_1$. This is the most important factor for the efficiency of multi-round SELEX.

Section III: Stochastic Model

- Sketch of proof for the optimal policy:
- If the current $\mathbb{A}_1$ proportion is r, then after one round of SELEX, the expected $\mathbb{A}_1$ proportion is at most

$$f(r) = r\bar{K}_1/[r\bar{K}_1 + (1-r)\bar{K}_2],$$

  which requires $A_1, A_2 \gg S$.
- Denote the $\mathbb{A}_1$ proportion after one round of SELEX as r_1 (small S) and r_2 (sufficiently large S). We have $\mathbb{E}r_1 = \mathbb{E}r_2$ and $\text{var}(r_1) > \text{var}(r_2) \approx 0$.
- $f(r)$ is is concave (downward). By Jensen's inequality, $\mathbb{A}_1$ proportion after two rounds of SELEX satisfies $\mathbb{E}f(r_1) < \mathbb{E}f(r_2)$.
- For multiple rounds of SELEX, the optimal policy should minimize the variance of r, meaning that $S \gg 1$.

Section III: Stochastic Model

- If $\mathtt{S} + \mathtt{A}_i \rightarrow \mathtt{SA}_i$ is much easier than $\mathtt{SA}_i \rightarrow \mathtt{S} + \mathtt{A}_i$, the binding can be regarded as irreversible.
- We stop the reaction when no more binding is possible (no free aptamer or no free target).
- In this irreversible situation, we cannot have the problematic case $a_1 = a_2 = 0$.
- When $S = 1$, $\mathbb{E}[a_1/(a_1 + a_2)] = A_1\bar{K}_1/(A_1\bar{K}_1 + A_2\bar{K}_2)$; when $S \geq A_1 + A_2$, $\mathbb{E}[a_1/(a_1 + a_2)] = A_1/(A_1 + A_2)$.
- $\mathbb{E}[a_1/(a_1 + a_2)]$ is strictly decreasing with S. Thus we have the same bounds as the reversible case.
- The optimal policy is to set $S = 1$.

Section IV: Summary

- We discuss SELEX, a process to select the best aptamer for binding a target.
- In the traditional deterministic model, the optimal policy (for any rounds of SELEX) is to have a very large aptamer number and a very small target number.
- We develop a stochastic model, in which the optimal policy for multiple rounds of SELEX is to have a very large aptamer number but a moderate target number.
- This theoretical analysis can be applied to other scenarios, such as selecting drug-resistant cells.

Reference

Wang, Y., Mistry, B. A., & Chou, T. (2022). Discrete stochastic models of SELEX: Aptamer capture probabilities and protocol optimization. The Journal of Chemical Physics, 156(24), 244103.