Analysis of Selection Approaches for Aptamer Molecular Libraries

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Aptamers are short strands of nucleic acids which fold into three dimensional structures having the ability to bind target molecules, such as proteins, small metabolites, or other nucleic acid structures. Aptamers are an emerging class of molecules which are being utilized in place of antibodies in a wide variety of applications, including protein purification, diagnostics, biosensor devices, and as pharmaceutical drug candidates. Central to the development of aptamers is to determine nucleic acid sequences which yield binding to desired target molecules with high affinity and specificity or with particular kinetic characteristics. Obtaining such aptamers poses a significant challenge in practice often requiring a lengthy and labor intensive refinement process in the laboratory. To identify key mechanisms by which selection may be made more efficient, we present mathematical analysis of several approaches which can be used for the refinement of aptamer libraries. We also discuss approaches for selection of aptamers having specified kinetic characteristics.

I. INTRODUCTION

Many approaches of molecular biology and biotechnology require the development of molecules which robustly bind specific target molecules. In molecular biology such recognition molecules are used in techniques such as fluorescently labeled affinity probes for proteins and metabolites [1, 2], protein purification [3], and inhibitors in metabolic and gene regulatory interactions [3, 4]. In biotechnology and pharmaceutical applications such recognition molecules are used as affinity probes in diagnostic assays [5, 6], recognition elements in biosensors [7, 8], and inhibitors in target validation in drug development [3, 4, 9, 10], and in theuropeutics [11–13].

Aptamers are a promising class of molecules for use in these application areas. Aptamers consist of short strands of nucleic acids which are capable of binding a very wide class of molecules, including proteins, small metabolites, and even other nucleic acid structures [1, 14, 15]. Aptamers are particularly promising since they are stable under a variety of ionic, pH, and thermal conditions, and can often be readily chemically modified for immobilization on substrates often with little change in specificity and binding strength [1, 15]. Aptamers can also be readily synthesized in quantity in the laboratory through Polymerase Chain Reactions (PCR). In practice, a key challenge is in identifying an appropriate nucleic acid sequence to achieve binding to a particular target molecule with high affinity and specificity or with specific binding kinetics. In this paper we shall give a mathematical analysis of several approaches proposed for the identification of aptamer nucleic acid sequences.

The primary approach considered starts with a library of aptamer molecules having random nucleic acid sequences. The initial library is then exposed to the target molecules. The unbound aptamers are removed and the bound aptamers are eluted from the substrate surface for amplification by PCR. In principle, this results in a new library of molecules consisting only of aptamers which previously were bound to the substrate surface. This process is repeated with the new library exposed to the target molecules and aptamers amplified by PCR to obtain successively more refined libraries.

Ideally, during each round of selection and amplification the population of the strongest binding aptamers increases as a fraction of the newly obtained library. This cycle is repeated until the strong-binding aptamers sufficiently dominate the molecular library. This approach is referred to as Systematic Evolution of Ligands by Exponential

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Enrichment (SELEX), originating in the work of [16–19]. To identify the key features of this approach we develop a mathematical model of the refinement process in Section II. An analysis of the convergence of this iterative refinement process is discussed in Section II D 2.

This widely used approach often requires many labor intensive rounds of selection to yield strongly binding aptamers. In practice, there are also many factors which influence the efficiency of selection. One important issue concerns the purity with which the selection step can be carried out. A potential source of trouble arises from aptamers which poorly bind the target molecules being retained in the selection step and being amplified by PCR. This can occur as a consequence of non-selective binding to the underlying substrate on which the target molecules are immobilized. We investigate approaches to reducing the presence of non-selective aptamers and poor binders in the refined molecular library in Section II F.

Another important factor is the critical role played by the concentration of target molecules exposed to the aptamer library. By choosing sufficiently small concentrations of the target molecules, one can achieve a strong level of competition between the aptamers molecules for binding a target molecule. The role of competitive binding effects in the limit of small target concentrations is investigated in Section II D 2.

To obtain aptamers having greater specificity and to enhance the selection step, we also investigate approaches in which a second competing target species is introduced. When the second target is chosen to have a large concentration relative to the library concentration and primary target species, it is shown that selectivity can be significantly enhanced. The second target serves to filter from the many library non-selective aptamers and also those which bind both target species. Analysis of this approach is discussed in Section II E.

The recent introduction of microfluidic devices allows for the possibility of automation and a more refined treatment of many of the experimental steps used in aptamer selection [20–23]. We discuss one approach based on the immobilization of target molecules on the surface of micromagnetic beads and processing using microfluidics [20]. We discuss an approach for selecting aptamers with specified binding kinetics in Section III.

The identification of aptamers for specific target molecules poses a set of interesting and challenging problems. The mathematical modeling, analysis, and simulations presented here aims to offer insights into the fundamental principles underlying experimental approaches to aptamer selection.

II. SYSTEMATIC EVOLUTION OF LIGANDS BY EXPONENTIAL ENRICHMENT (SELEX)

A widely used approach for the development of aptamers is the Systematic Evolution of Ligands by Exponential Enrichment (SELEX), originating in the work of [16–19]. In SELEX an initial library consisting of molecules having random nucleic acid sequences is exposed to target molecules, typically immobilized on a substrate. After an incubation period the substrate is then washed to remove unbound aptamers. The aptamers bound to target are then eluted into the buffer solution. The eluted aptamers are then amplified by cycles of Polymerase Chain Reactions (PCR). The resulting library of aptamer molecules is then exposed again to the target molecules. This process is repeated to obtain a refined library of aptamers for the target molecules.

Typically, the selection step in which aptamers are exposed to target is allowed to reach thermodynamic equilibrium. This results in the selection favoring aptamer molecules which bind strongly to target molecules, as characterized by the dissociation constant. Ideally, as the SELEX cycle is repeated the strongest binding aptamers increasingly dominate the molecular library. For recent reviews of SELEX and related variants see [24–27].

To gain insights into the basic mechanisms underlying SELEX, we develop a mathematical model of this selection procedure. Initially, we model the chemistry of SELEX in terms of mass-action kinetics. This is expected to be applicable in the regime where there is a large number of reagent molecules which are spatially well-mixed. Other variants could also be considered in which SELEX is carried out only with a small number of molecules.

A. Mass-Action Chemical Kinetics

To model the SELEX procedure the aptamers of the library are divided up into distinct classes. The aptamer classes are distinguished by the kinetic rate constants for the aptamers to bind and unbind the target species. Let $[A_i]$ denote the concentration of freely diffusing unbound aptamers of type i. Let $[S]$ denote the concentration of exposed unbound target sites. The concentration of aptamers of type i which are bound is denoted by $[A_i S]$. The aptamer and target binding and unbinding events are modeled for the library by the following mass-action kinetics

$$[A_i] + [S] \underset{k_{\rm off}^{(i)}}{\overset{k_{\rm on}^{(i)}}{\rightleftharpoons}} [A_i S]. \tag{1}$$

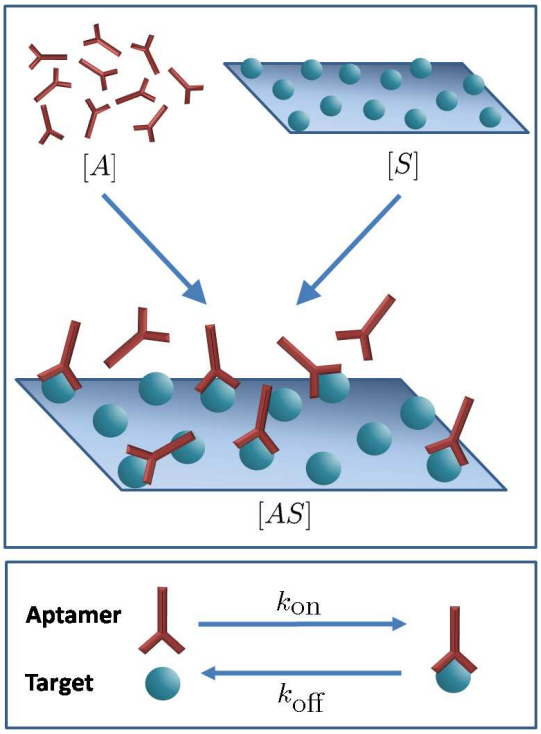

FIG. 1: Aptamer Library Exposed to Target Molecules Immobilized on a Substrate. Aptamers are exposed to target molecules immobilized on a substrate. A stoichiometry is assumed allowing only for one aptamer to bind at a time to a target molecule.

The $k_{\text{on}}^{(i)}$ denotes the second order kinetic rate for an aptamer binding to available target molecules. The $k_{\text{off}}^{(i)}$ denotes the first order rate for an aptamer unbinding from a target molecule. The aptamer selection step and kinetics are illustrated in Figure 1.

In the kinetics, a stoichiometry is assumed in which only one aptamer molecule may bind at a time to a target molecule. Under this assumption the aptamers and target have concentrations which evolve according to

$$\frac{d[A_i]}{dt} = -k_{\text{on}}^{(i)}[A_i][S] + k_{\text{off}}^{(i)}[A_iS] \tag{2}$$

$$\frac{d[S]}{dt} = -\sum_i k_{\text{on}}^{(i)}[A_i][S] + \sum_i k_{\text{off}}^{(i)}[A_iS]. \tag{3}$$

The total concentration of target is given by

$$[S_T] = [S] + \sum_i [A_iS]. \tag{4}$$

The total concentration of aptamers of type i, irrespective of whether it is bound or un-bound, is given by

$$[A_{T,i}] = [A_i] + [A_iS]. \tag{5}$$

The total target concentration of equation 4 and total aptamer concentrations of equation 5 are conserved quantities remaining constant throughout the selection step. The aptamer-target dissociation constant for each aptamer class is denoted by

$$k_d^{(i)} = k_{\text{off}}^{(i)}/k_{\text{on}}^{(i)}. \tag{6}$$

The average of the dissociation constants of the aptamers which adhere to the target molecules is given by

$$\bar{k}_d = \sum_i \frac{[A_{T,i}S]}{[A_TS]} k_d^{(i)}. \tag{7}$$

The $[A_TS]$ denotes the total concentration of bound aptamers irrespective of their type.

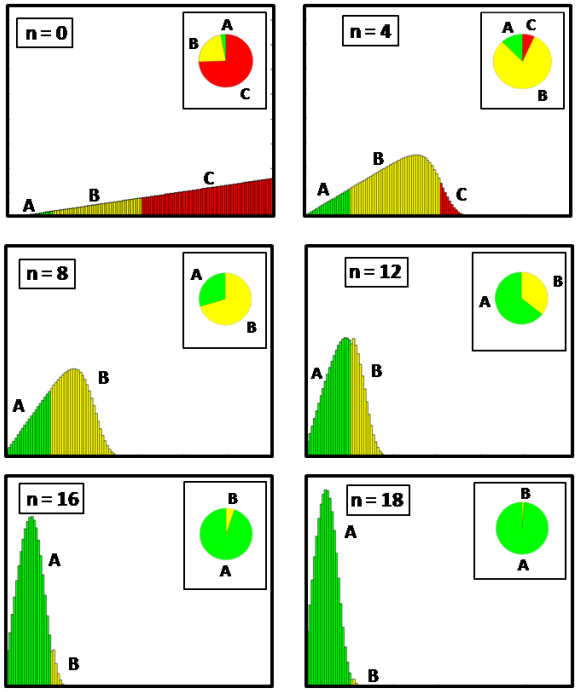

FIG. 2: Refinement of the Aptamer Library. After successive rounds of refinement the fraction of the library comprised of each aptamer type is shown. Three aggregate classes are also labeled A, B, C, corresponding respectively to binders having picoMolar, nanoMolar, or greater than microMolar dissociation constants. The inset pie chart shows the percentage of binders of each of these aggregate classes. Shown are the rounds $n = 0, 4, 8, 12, 16, 18$. The 18th round is the first time purity for picoMolar binders achieves a level above 99%. Parameters values for the aptamer library and target species are given in Table II.

B. Thermodynamic Equilibrium : Steady-State Mass-Action Kinetics

Thermodynamic equilibrium of the system corresponds in this case to the steady-state of the kinetic equations 2-3. At steady-state the dissociation constant can be expressed as

$$k_d^{(i)} = \frac{[A_i][S]}{[A_i S]}. \tag{8}$$

and the bound aptamer concentration can be expressed as

$$[A_i S] = \frac{[S][A_{T,i}]}{k_d^{(i)} + [S]} \tag{9}$$

These expressions follow from setting to steady-state equation 2- 3 and using equation 5. The dissociation constant averaged over the population of bound aptamers is given by

$$\bar{k}_d = \frac{[A][S]}{[AS]} \tag{10}$$

$$[AS] = \frac{[S][A_T]}{\bar{k}_d + [S]}. \tag{11}$$

This gives an implicit equation for the steady-state concentration of target molecules which are not bound to any aptamers

$$[S]\left(1 + [A_T]\sum_j \frac{F_j}{k_d^{(j)} + [S]}\right) = [S_T]. \tag{12}$$

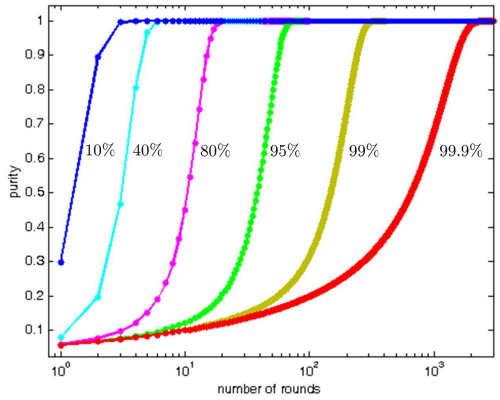

FIG. 3: Purity of the Aptamer Library for Successive Selection Rounds. The purity for picoMolar binders in the aptamer library is shown after each round of selection. The labeled percentages show selection for different ratios $[S_T]/[A_T]$. Parameter values for the aptamer library and target species is given in Table III.

The fraction of the library comprised of aptamers of a given type is denoted by

$$F_i = \frac{[A_{T,i}]}{[A_T]}. \tag{13}$$

A mass-action kinetics model and analysis of the steady-state similar to our initial analysis of SELEX was also proposed in [28].

C. Analysis of the Selection Step

In each round of the SELEX method, there is an attempt to retain only aptamers which bind to the target molecules. This is used to filter the aptamer population by favoring aptamers with the lowest dissociation constant k_d for the target. Two primary variables under experimental control are the initial concentration of the aptamer library $[A_T]$ and the initial concentration of the target species $[S_T]$. We investigate general features of the selection step as these initial concentrations are varied. We then present results showing how optimal selectivity can be attained within the physical regime for which the mass-action kinetics model is valid.

In Figure 2 refinement of the library by successive rounds of selection is shown when $[S_T]/[A_T] = 0.8$. In successive rounds the aptamer library is found to be enriched in aptamers which bind more strongly to the target molecules. In Figure 3, it is shown how the purity of the library increases after successive rounds of selection and how this changes as the ratio $[S_T]/[A_T]$ is varied. The number of rounds required to reach 99% purity for picoMolar binders is shown in Figure 4. It is found that as the ratio $[S_T]/[A_T]$ becomes small the number of rounds required to reach a high level of purity greatly decreases.

To analyze the selection step, we characterize the library using the average dissociation constant. From equations 7-11, this can be expressed as

$$\bar{k}_d = \sum_i p_i k_d^{(i)}, \text{ with } p_i = \left(\frac{[A_{T,i}]}{[A_T]}\right)\left(\frac{\bar{k}_d + [S]}{k_d^{(i)} + [S]}\right). \tag{14}$$

To investigate the role of the target species concentration, it is useful to consider two extreme cases. The first is the case in which target molecules are plentiful. This corresponds to the limit $[S_T]/[A_T] \to \infty$. The second is the case in which the target molecules are scarce. This corresponds to the limit $[S_T]/[A_T] \to 0$. From equation 12 these cases also correspond respectively to $[S]/[A_T] \to \infty$ and $[S]/[A_T] \to 0$.

We first consider the limit $[S]/[A_T] \to \infty$. In this case, $p_i \to p_i^* = [A_{T,i}]/[A_T]$, since $\bar{k}_d \leq \max k_d^{(i)} < \infty$. This gives for the average dissociation constant of the bound aptamers

$$\bar{k}_{d,\infty} = \sum_i \left(\frac{[A_{T,i}]}{[A_T]}\right) k_d^{(i)}. \tag{15}$$

In this rather extreme case the target is so plentiful it absorbs all of the aptamer molecules in proportion to their representation in the initial aptamer library. This shows that the selection step which produces a new library from the bound aptamers is no better than the initial library subject to selection. Consequently, there is no improvement of the dissociation constant of the population over the selection step.

We now consider the second case where the target is scarce corresponding to $[S]/[A_T] \to 0$. From equations 9-10 the average dissociation constant can be expressed as

$$\bar{k}_d + [S] \;=\; \frac{[A_T][S]}{[AS]} = \left[\sum_i \frac{[A_{T,i}]}{[A_T]} \left(k_d^{(i)} + [S]\right)^{-1}\right]^{-1} \tag{16}$$

where we use that $[AS] = \sum_i [A_i S]$. In the limit $[S]/[A_T] \to 0$ we have

$$\bar{k}_{d,0} \;=\; \left[\sum_i p_i^* \left(k_d^{(i)}\right)^{-1}\right]^{-1} \tag{17}$$

where $p_i^* = [A_{T,i}]/[A_T]$ as before. By comparing equation 15 and 17 it can be shown that $\bar{k}_{d,0} < \bar{k}_{d,\infty}$ holds for any choice of physical parameters, see Appendix A 1.

This shows that the selection step produces a new library from the bound aptamers which has a better dissociation constant on average than the initial library. This is a consequence of the competition which occurs between the aptamers of the library in binding available target molecules which are now scarce.

While the above represents rather extreme cases, in practice, the target molecules will likely be present in numbers corresponding to an intermediate concentration. From equation 14, it can be shown that $\partial \bar{k}_d/\partial[S] > 0$ so that the average $\bar{k}_d$ decreases monotonically as the available target is decreased, see Appendix A 2. This monotonic behavior is also seen in the successive refinements of the aptamer library in Figures 2 and 3.

This shows that as $[S]/[A_T]$ decreases the competitive binding effects always result in a decrease of the average dissociate constant associated with the aptamers which adhere to the target. This has as an important consequence that optimal single step selectivity is approached when the aptamer library is exposed to as little target as possible. This of course requires considering the physical regime in which the mass-action kinetics model is an appropriate description. A lower bound on the achievable average dissociation constant in a single step of selection is given by $\bar{k}_{d,0}$ of equation 17.

The analysis also suggests taking the initial concentrations of target and aptamers so that $[S_T]/[A_T]$ is as small as possible. The analysis suggests this will achieve purity for the strongly binding aptamers in the fewest number of rounds of selection, see Figure 4.

D. Analysis of SELEX Iterations

In each round of SELEX the new aptamer library is obtained from the aptamers which adhere to the target molecules. From the analysis above, the distribution of aptamer types in the new library is given by

$$B_i = \frac{[A_i S]}{[AS]} = \left[\sum_j \frac{k_d^{(i)} + [S]}{k_d^{(j)} + [S]} \left(\frac{F_j}{F_i}\right)\right]^{-1}. \tag{18}$$

The B_i is the fraction of type i aptamers in the new library. The F_i is the fraction of type i aptamers in the initial library subject to selection, as defined in equation 13. Using the average dissociation constant of the new library $\bar{k}_d$, this can also be expressed as

$$B_i = \frac{\bar{k}_d + [S]}{k_d^{(i)} + [S]} F_i. \tag{19}$$

This shows that over the selection step, those aptamers of type i which have dissociation constants smaller than $\bar{k}_d$ will comprise a greater fraction of the new library (since the prefactor multiplying F_i will be greater than one) and those with dissociation constants smaller than $\bar{k}_d$ will comprise a smaller fraction. The concentration $[S]$ of free target molecules controls the sensitivity of this dependence.

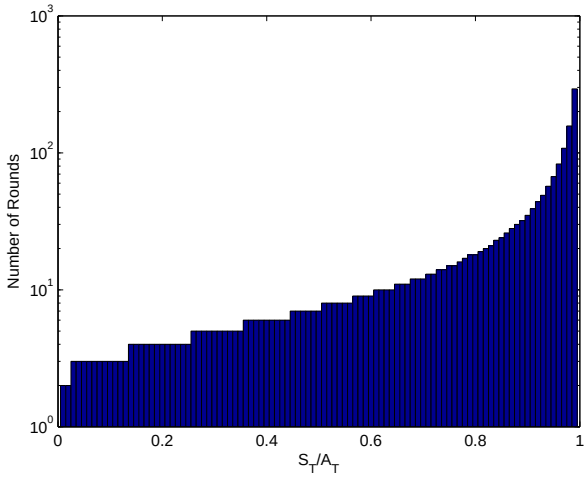

FIG. 4: Number Rounds of Required to Purify the Library as the Target Concentration is Varied. Shown are the number of rounds required to obtain a refined aptamer library with 99% purity for picoMolar binders as the ratio $[S_T]/[A_T]$ is varied. Parameter values for the aptamer library and target species is given in Table III.

Let $B_i^{[n]}$ denote the fraction of the library composed of aptamers of type i after the n^{th} selection step. From equation 19 the aptamer distribution yielded by a round of SELEX can be expressed as

$$B_i^{[n+1]} = \frac{\bar{k}_d^{[n+1]} + [S]}{k_d^{(i)} + [S]} B_i^{[n]}.$$

We now discuss what occurs in successive iterations depending on the concentration of target molecules.

1. *Regime of Saturating Target Concentration*

In the regime where target molecules are plentiful, corresponding to $[S] \gg k_d^{(i)}$ for all i, we can express the distribution of aptamers to leading order as

$$B_i^{[n+1]} = \left(1 + \frac{\bar{k}_d^{[n+1]} - k_d^{(i)}}{[S]}\right) B_i^{[n]}.$$

While in this regime aptamers with smaller dissociation constants are favored, the prefactor is very close to one and the selection step does not refine effectively the aptamer population each round. In practice, such a SELEX procedure would require many iterations to converge sufficiently to a library dominated by strong binding aptamers, see Figure 3 and 4.

2. *Regime of Low Target Concentration*

We now discuss the regime in which the target molecules are scarce relative to the concentration of aptamer molecules. We shall consider the specific regime $[S] \ll k_d^{(i)}$ for all i. In this regime the concentration of free aptamers will far exceed that of bound aptamers for each type. This follows from equation 8 which gives $[S]/k_d^{(i)} = [A_i S]/[A_i]$ which implies that $[A_i] \gg [A_i S]$.

To leading order, we have in this regime

$$B_i^{[n+1]} = \frac{\bar{k}_d^{[n+1]}}{k_d^{(i)}} B_i^{[n]}. \tag{20}$$

The average dissociation coefficient can be expressed as

$$\bar{k}_d^{[n+1]} = \left[\sum_i B_i^{[n]} \left(k_d^{(i)}\right)^{-1}\right]^{-1}. \tag{21}$$

Aptamers with small dissociation constants relative to $\bar{k}_d$ yield prefactors greater than one while aptamers with larger dissociation constants yield prefactors smaller than one. In practice the dissociation constants may vary over many orders of magnitude, resulting in significant refinement of the library population each selection step.

We now discuss convergence in this regime of the SELEX procedure to the best binding aptamer. This corresponds to $B_1^{[n]} \to 1$ and $B_i^{[n]} \to 0, i \geq 2$. It is convenient to work with the mathematically equivalent requirement for convergence that $1/B_1^{[n]} - 1 \to 0$. The fraction of the best binder can then be expressed and bounded by

$$\begin{aligned}\frac{1}{B_1^{[n]}} - 1 = \frac{1 - B_1^{[n]}}{B_1^{[n]}} = \frac{\sum_{j=2}^N B_j^{[n]}}{B_1^{[n]}} = \frac{\sum_{j=2}^N \frac{k_d^{(1)}}{k_d^{(j)}} B_j^{[n-1]}}{B_1^{[n-1]}} \\ \leq \left(\frac{k_d^{(1)}}{k_d^{(2)}}\right)\left(\frac{1 - B_1^{[n-1]}}{B_1^{[n-1]}}\right) \leq \left(\frac{k_d^{(1)}}{k_d^{(2)}}\right)^n \left(\frac{1 - B_1^{[0]}}{B_1^{[0]}}\right).\end{aligned}$$

We use the convention that $k_d^{(i)}$ are indexed in increasing order. Since $k_d^{(1)}/k_d^{(2)} < 1$ we have as $n \to \infty$ that $B_1^{[n]} \to 1$ and $\bar{k}_d \to k_d^{(1)}$. This shows that in the regime of small target concentration the SELEX procedure converges to an aptamer population consisting of the best binding aptamer.

Besides using as small a concentration of target as possible, the selection step might be further improved by pursuing additional forms of selectivity each round of refinement. In practice, there is also the potential for contaminants on to degrade selectivity each round. We now discuss strategies by which SELEX might be further enhanced.

E. Enhancement of the Selection Step Utilizing Competing Target Species

One approach to enhance the selective step is to introduce an additional target species in large concentration to compete with the primary target species. The basic strategy is predicated on the assumption that many weakly binding aptamers will likely be non-selective. As a consequence, many of these aptamers will bind to both the primary and secondary target species. Since the secondary target species has a much larger concentration than the primary target species, many of these weakly binding aptamers will be removed from the library during a round of such selection. This will retain in the library those aptamers which are more selective for the primary target molecules. This approach to selection is illustrated in Figure 5.

This approach could be carried out with multiple target species. We consider only the specific case in which there are two target species, the primary one present in only a small concentration while the secondary is present in large concentration.

For notational convenience, let the concentrations considered before be superscripted by (a) for the primary target species and (b) for the secondary target species. For example, $[S^{(a)}]$ will now denote the concentration of unbound target molecules of the primary target species and $[S^{(a)}]$ will denote similarly for the secondary species. The other concentrations and dissociation constants are treated with the same notational convention.

At thermodynamic equilibrium (steady-state) the bound populations for each aptamer and target type are given by

$$\begin{aligned}\left[A_i S^{(a)}\right] &= \frac{[A_{T,i}][S^{(a)}]k_d^{(i),(b)}}{([S^{(a)}] + k_d^{(i),(a)})([S^{(b)}] + k_d^{(i),(b)}) - [S^{(a)}][S^{(b)}]} \\ \left[A_i S^{(b)}\right] &= \frac{[A_{T,i}][S^{(b)}]k_d^{(i),(a)}}{([S^{(a)}] + k_d^{(i),(a)})([S^{(b)}] + k_d^{(i),(b)}) - [S^{(a)}][S^{(b)}]}.\end{aligned}$$

These expressions follow similarly to Section II A using the total aptamer concentration $[A_{T,i}] = [A_i] + [A_i S^{(a)}] + [A_i S^{(b)}]$.

As discussed in SectionII D the selection step performs best when the desired target concentration is as small as possible. To simplify the analysis we shall consider the regime where the desired aptamer concentration is sufficiently

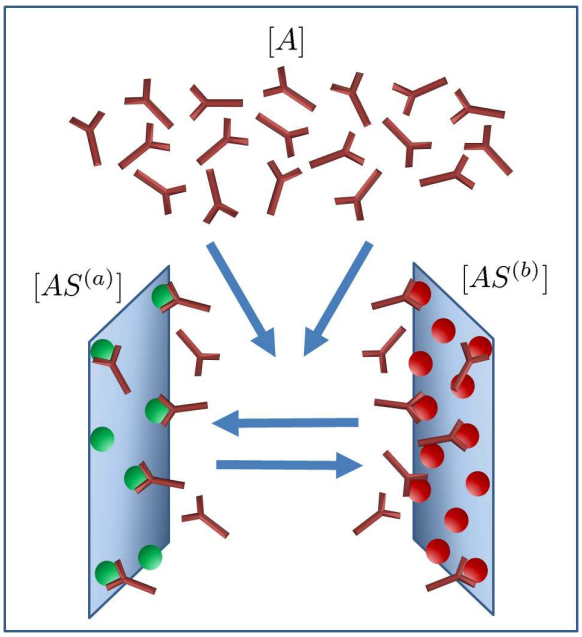

FIG. 5: Selection using Multiple Target Species. To further enhance selectivity during each refinement round the aptamer library is exposed to both a primary and secondary target species. For the secondary target species many more molecules relative to the primary target species are presented to the library to deplete the library of aptamers which bind non-selectively.

small so that $[S^{(a)}] << k_d^{(i),(a)} \left(1 + \frac{[S^{(b)}]}{k_d^{(i),(b)}}\right)$ for all i. Since the best binder for the desired target is expected to be highly selective we shall also assume that it binds more poorly to the competing target than all other aptamers, $k_d^{(1),(b)} \geq k_d^{(i),(b)}$ for all i.

In this regime, the SELEX iterations yield population fractions given by

$$\frac{B_i^{[n+1]}}{B_1^{[n+1]}} = \left[\left(\frac{k_d^{(1),(a)} + [S^{(a)}]}{k_d^{(i),(a)} + [S^{(a)}]}\right)\Gamma^{(i)}\right]\left(\frac{B_i^{[n]}}{B_1^{[n]}}\right) \tag{22}$$

$$= \left[\left(\frac{k_d^{(1),(a)} + [S^{(a)}]}{k_d^{(i),(a)} + [S^{(a)}]}\right)\Gamma^{(i)}\right]^{n+1}\left(\frac{B_i^{[0]}}{B_1^{[0]}}\right) \tag{23}$$

where

$$\Gamma^{(i)} = \frac{1 + [S^{(b)}]/k_d^{(1),(b)}}{1 + [S^{(b)}]/k_d^{(i),(b)}}. \tag{24}$$

In the regime under consideration $\Gamma^{(i)} < 1$, $i \geq 2$ so that the refinement of the library over the selection step is enhanced by the introduction of the competing target species. The $\Gamma^{(i)}$ is a decreasing function of $[S^{(b)}]$ with $\Gamma^{(i)} \geq k_d^{(i),(b)}/k_d^{(1),(b)}$, so that $[S^{(b)}]$ should be chosen sufficiently large to realize the full potential of this effect. The improvement in the selection step is especially pronounced for aptamers which bind the competing target species significantly better than the sought best binder for the desired target species binds the competing target molecules, $k_d^{(i),(b)} \ll k_d^{(1),(b)}$.

The analysis above shows that $B_i^{[n]} \to 0$, $i \geq 2$, as $n \to \infty$ under the assumptions of the regime considered. Since $\sum_i B_i^{[n]} = 1$, we find that the modified SELEX method converges to the best binding aptamer, $B_1^{[n]} \to 1$. From equation 23 we see this will be at a faster rate than the standard SELEX method. This is illustrated by the number of rounds required to purify the library with and without the secondary target species as shown in Figures 4 and 6.

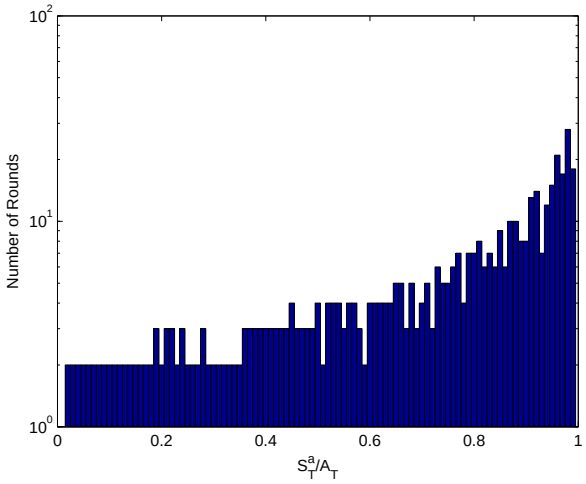

FIG. 6: Number Rounds of Required to Purify the Library for Multiple Target Species. Shown are the number of rounds required to obtain a refined aptamer library with 99% purity for picoMolar binders as the ratio $[S_T^{(a)}]/[A_T]$ is varied. During the selection round the aptamer library is also exposed to a secondary target species to deplete the library of non-selective binders. Parameter values for the aptamer library and target species is given in Table IV. The number of rounds required to achieve purity should be compared with Figure 4.

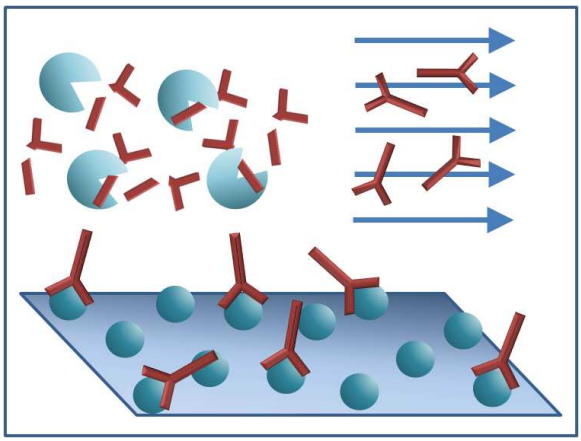

FIG. 7: Selection by Degradation of Unbound Aptamers. Degradation of aptamers which are unbound in the solution can be achieved either by specialized enzymes which bind and catalyze the destruction of aptamer molecules (top left) or by subjecting the target substrate to a clean buffer solution and fluid flow which carries off any unbound aptamers (top right). Such an enzymatic cleaning or washing process is expected to further enhance enrichment of the library for aptamers which bind strongly to the target substrate.

F. Enhancement of the Selection Step Utilizing Aptamer Degradation

Another approach by which selection from the library can be enhanced is to introduce enzymes which degrade aptamer molecules which are unbound and free in the solution. This strategy is predicated on a greater rate of degradation of aptamer types which spend a larger proportion of time free in the solution. In the case that target molecules are immobilized on a substrate, subjecting the library solution to drift by an electric field or fluid flow could also realize a similar effect, see Figure 7.

To study the role of such a process, we consider a system starting with a large concentration of aptamer molecules free in the solution, which then is reduced by the degradation process. This is a non-equilibrium state which is ultimately pushed toward an equilibrium (steady-state). Since the equilibrium corresponds to the state with all aptamers degraded, to be useful the process must be stopped before reaching complete equilibrium.

To illustrate the basic mechanism, we consider the regime in which target molecules are available in large concentration relative to the aptamer library. Similar effects are expected when the target species has a low concentration

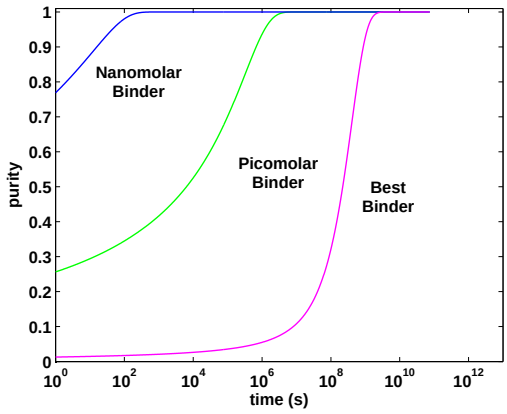

FIG. 8: Purity of Aptamer Library Subject to Degradation. The purity of a library refined over time strictly by degradation is shown. The level of purity of the library over time for nanomolar aptamers, picomolar aptamers, and the best binding aptamer of the initial library is shown. Parameters of the aptamer library, target species, and degradation process can be found in Table **??**.

relative to the aptamer library. In this case selection would be further enhanced by competitive binding effects. For sufficiently abundant target substrate $[S_T]$, the concentration of free target species can be treated as changing only negligably. In this regime, the chemical kinetics are given by

$$\frac{d}{dt}\begin{bmatrix}[A_i]\\ [A_{T,i}]\end{bmatrix} = \begin{bmatrix}-k_{\text{on}}^{(i)}[S_T]-k_{\text{off}}^{(i)}-\gamma & k_{\text{off}}^{(i)}\\ -\gamma & 0\end{bmatrix}\begin{bmatrix}[A_i]\\ [A_{T,i}]\end{bmatrix}. \tag{25}$$

where $[A_{T,i}] = [A_i] + [A_iS]$. We shall consider the process when approaching equilibrium (steady-state) which corresponds to $[A_{T,i}], [A_i] \to 0$ as $t \to \infty$. By solving these linear equations as equilibrium is approached, the fraction of aptamers of type i bound to the target at time t can be approximated by

$$B_i(t) = \frac{p_+^{(i)} e^{\lambda_+^{(i)} t}}{\sum_j p_+^{(j)} e^{\lambda_+^{(j)} t}}. \tag{26}$$

The λ_+ is the eigenvalue of the linear system which is smaller in magnitude. The p_+ is a coefficient depending on the initial aptamer library concentrations. For more details of how this expression was derived, see Appendix B.

In the regime where $[S_T] \gg k_d^{(i)} + \gamma/k_{\text{on}}^{(i)} + \sqrt{k_d^{(i)}\gamma/k_{\text{on}}^{(i)}}$ we have $\lambda_+^{(i)} = -\gamma k_d^{(i)}/[S_T]$. Since $k_d^{(1)} < k_d^{(i)}$, $i \geq 2$ we have from equation 26 that $B_i(t) \to 0$, $i \geq 2$ and $B_1(t) \to 1$. This shows that the aptamer species with smallest dissociation constant will be favored by the degradation based selection step. Over time the aptamer with the smallest dissociation constant will increasingly dominate the aptamer population bound to target molecules, see Figure 8.

III. MICROFLUIDIC SELECTION

SELEX is often carried out by immobilizing target molecules on a substrate. The substrate is then exposed to the aptamer library solution and washed to remove unbound aptamers. The bound aptamers are then eluted from the target molecules and amplified by PCR for another round of selection or for sequencing (cite).

In practice, if the target molecules are immobilized on a substrate having a large surface area the aptamers may bind to the substrate or surface contaminant instead of the target molecules. This is expected to result in a weakening of the selective pressure and efficiency of the selection step each round of SELEX. To help reduce such effects, target molecules can be immobilized on micron sized beads, which offer a low surface-to-volume ratio. The beads could also be prepared so that the bare surface has a net negative charge to inhibit the non-selective binding of aptamers to the bead surface, see Figure 9.

The use of micron beads also provides a mean by which to achieve a very small effective concentration for the target species exposed to the aptamer library, thereby benefiting from competitive binding effects as discussed in Section II D.

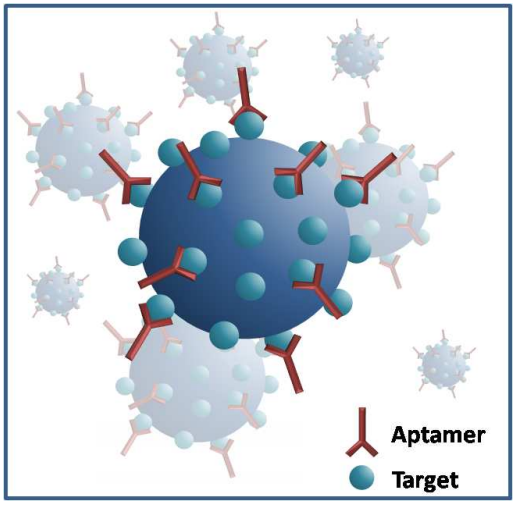

FIG. 9: Target Molecules Immobilized on Micromagnetic Beads. To achieve very small concentration of target to expose to the aptamer library, target molecules are immobilized on the surface of micron sized beads. The use of micron scale beads offers a low surface-to-volume ratio of the exposed substrate to the library solution and reduces the chance for significant surface contaminants and non-selective aptamer binding to the substrate. The use of beads fabricated of a material having a magnetic moment can also be used to facilitate experimental manipulation.

The use of beads made of a material having a magnetic moment could also be used to faciliate manipulation of the target species and substrate by magnetic tweezers or microfluidic devices (cite).

We now discuss approaches to aptamer selection which utilize microfluidics to manipulate micromagnetic beads on which target molecules are immobilized. In particular, we discuss an approach by which a microfluidic device could be utilized to select aptamers with small k_d and specified kinetics. We consider approaches to select for one of two different types of kinetics which may be of interest in applications.

The first type of kinetics corresponds to aptamers with small k_d and has a small k_{off}. This type of kinetics corresponds to aptamers which bind a target at a low rate but once attached remain bound for a long period of time.

The second type of kinetics corresponds to aptamers with small k_d and with large k_{off}. This type of kinetics corresponds to aptamers which bind a target at a high rate but also detach after a relatively short period of time. For applications favoring selective but transient binding this class of aptamer molecules may be of particular interest (cite).

To carry out the selection process the beads are processed by a microfluidic device. The microfluidic device consists of a wide channel having a thin depth and with pre-patterned magenetic strips along the channel bottom. Feeding into the channel are several inlets through which fluid is injected and several outlets from which fluid is collected. The strips are designed to exert a local magnetic field on the beads so that as they are carried by the flow they migrate toward the channel center, see Figure 10.

The inlets are chosen to alternate between clean buffer and an aptamer library solution. The beads are initially introduced into the device in the first and last clean buffer inlets. The fluid flow of the buffer carries the beads into the microfluidic channel.

When a bead encounters a magnetic strip the combination of local magnetic forces and hydrodynamic drag forces act to move the bead toward the channel center, see Figure 12. A bead is processed by the microfluidic device in effectively three stages. In the first stage, the bead introduced into the clean buffer encounters the magnetic strip and migrate toward the center of the channel. In the second stage, the bead enters the aptamer solution exposing the target molecules to the aptamer library. In the third stage, the bead leaves the aptamer library solution and again enters a clean buffer solution near the center of the channel. In the third stage, the bead also disassociates from the magnetic strip and is collected for processing. These three processing stages are shown in Figure 10. The forces acting on the beads driving the migration toward is channel center is shown in Figure 12 and discussed in more detail in [20].

In this microfluidic approach there are primarily two time scales which can be controlled. The first is the duration of time t_1 the bead spends exposed to the aptamer library solution. The second is the duration of time t_2 after exposure the bead spends in the clean buffer before being collected and processed. In principle, these time scales can be controlled by the length and angle of the magnetic strips, by the rate of fluid flow, size of the beads (hydrodynamic drag), and by the width of the different fluid domains (clean buffer/ aptamer library solution).

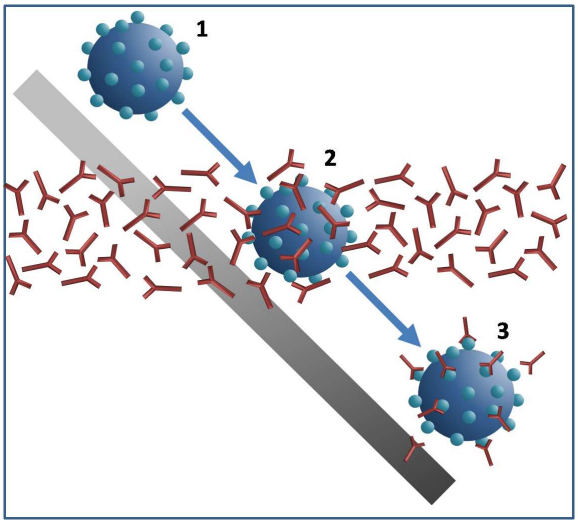

FIG. 10: Microfluidic Device for Processing Micromagnetic Beads on which Target is Immobilized. A bead is processed by the microfluidic device in three stages. In the first stage, a bead with immobilized target on the surface is introduced into the clean buffer and when encountering a magnetic strip migrates toward the channel center. In the second stage, the bead moves toward the channel center and encounters the aptamer library. In the third stage, as the bead continues moving toward the channel center it again enters a clean buffer solution. The time scales associated with each stage can be controlled, in principle, by varying the rate of the fluid flow, bead radius (hydrodynamic drag), length of the magnetic strips, and size of the different fluid domains (clean buffer/aptamer solution).

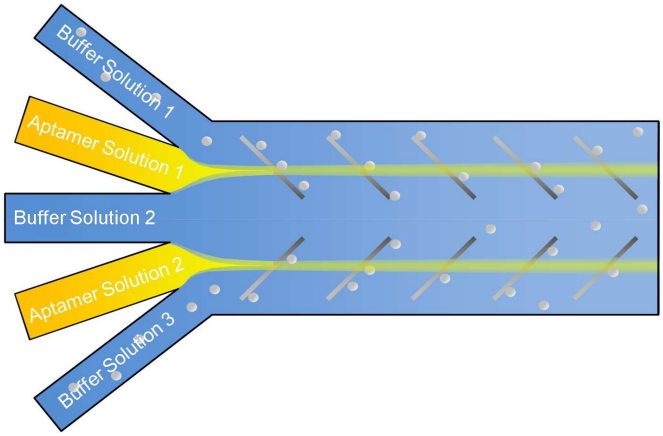

FIG. 11: Microfluidic Device and Flow Configuration. The microfluidic device is configured with alternating buffer solution and a solution containing the aptamer library. Patterned along the bottom of the channel are magnetic strips which exert a local magnetic field on the beads within the channel. When a bead encounters a magnetic strip it migrates toward the channel center passing through the aptamer library and exiting into the clean buffer in the center. The beads and buffer solution in the center are collected by an outlet in the channel center for further processing and analysis (not shown).

By adjusting these time scales, selection can be tuned for specified kinetic characteristics. To illustrate the basic mechanism, we consider a regime in which the time scales of binding and dissociation are well separated. This is consistent with aptamers having a low k_d. More precisely, we assume the average time for an aptamer to bind a target and the average time for an aptamer to dissociate from a target satisfies $1/k_{\text{on}}^{(i)} \leq \alpha \ll \beta \leq 1/k_{\text{off}}^{(i)}$, where α, β can be chosen so this holds for any choice of i.

To select aptamers satisfying the first type of kinetics discussed above in which aptamers bind for long duration, the bead is exposed to the aptamer solution for a time t_1 which is sufficient for thermodynamic equilibrium (steady-state) to be achieved. In this case, the fraction of the aptamer library bound to target is given after exposure by

$$B_i(t_1) \;=\; \frac{\bar{k}_d}{k_d^{(i)}} F_i. \tag{27}$$

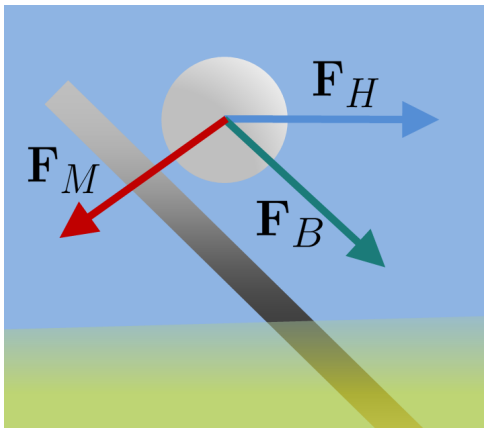

FIG. 12: Forces Acting on the Micromagnetic Beads. Two primary forces act on the micromagnetic beads. The first arises from the fluid flow and hydrodynamic drag on beads denoted by F_H. The second arises from the local magnetic field of the pre-patterned strips and is denoted F_M. The net force which acts on the bead is denoted by F_B, which moves the bead down the strip toward the channel center.

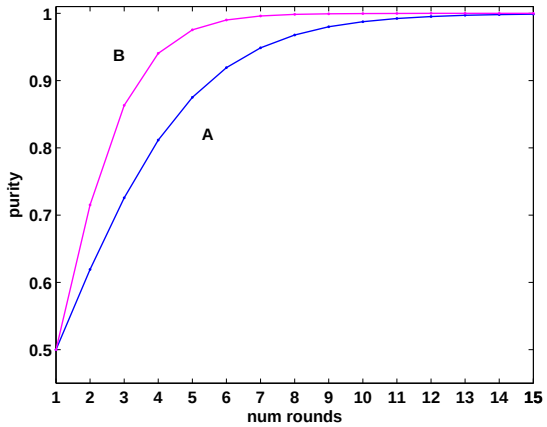

FIG. 13: Purity of Aptamers Selected with Kinetic Characteristics. The purity of the aptamer library for each of the kinetic classes is shown. The curve labelled A shows the purity of the library for aptamers which have the first type of kinetics, in which aptamers bind target for long duration. The curve labelled B shows the purity of the library for aptamers which have the second type of kinetics, in which aptamers bind target molecules only transiently. In both cases the aptamers having specified kinetics are selected from a library consisting only of aptamers having a low dissociation constant k_d.

The F_i is the fraction of the library which consists of aptamers of type i. These expressions follow from the analysis given in Section (ref) and equation (ref).

To select aptamers having the second type of kinetics discussed above in which aptamers bind only transiently, the bead is exposed to the aptamer solution for a duration of time t_1 which is sufficiently small to filter-out aptamers having large $k_{\rm on}$. This time is also taken to be smaller than the time it takes for dissociation to occur, $t_1 \leq \alpha$. This ensures over the exposure time $[0, t_1]$ that only a negligible number of aptamers bind a target and then dissociate. In this case, the fraction of bound aptamers which are of type i is given to leading order by

$$Q_i(t_1) \;=\; \frac{k_{\rm on}^{(i)} F_i}{\sum_j k_{\rm on}^{(j)} F_j}. \tag{28}$$

The F_i is the fraction of the library which consists of aptamers of type i.

After exposure to the aptamer library solution in each of these cases the bead continues its migration down the magnetic strip and into a clean buffer solution. When the bead reaches the end of the magnetic strip it is swept by the flow to the center collection outlet. During this time the bound aptamers may dissociate from the target molecules on the bead surface. We shall denote the duration of time from when the bead exits the band of fluid of the library solution and is collected for processing by t_2.

To illustrate how this step of the microfluidic process can be utilized for selection we consider the case where the bead spends only a short duration of time in the clean buffer solution before being collected $t_2 \ll \min_j 1/k_{\text{off}}^{(j)}$.

To select aptamers meeting the first criteria, the protocol will be utilized to try to select aptamers which have a time of dissociation which is on average greater than t_2. In this regime the fraction of aptamers of type i which are bound to the bead surface at time t_2 is given to leading order by

$$B_i(t_1 + t_2) = \frac{B_i(t_1)\left(1 - k_{\text{off}}^{(i)} t_2\right)}{\sum_j B_j(t_1)\left(1 - k_{\text{off}}^{(j)} t_2\right)} \tag{29}$$

where $k_{\text{off}}^{(i)}$ is the effective single molecule kinetic rate of unbinding.

This shows that the duration of time spent by the bead in the clean buffer results in the selection process favoring aptamers with small dissociation kinetic rates (long time for dissociation), see equation 29.

To select the aptamer meeting the second criteria, the protocol can be used to obtain aptamers which have good binding properties, small k_d, but large k_{on} and k_{off}. This is achieved by exposing the bead to the aptamer library for a relatively brief period t_1 followed by amplify the aptamers found present in the surrounding buffer solution of the bead when collected in the nearly clean buffer solution. In this case, the fraction of free aptamers in the buffer solution which are of type i is given to leading order by

$$Q_i(t_1 + t_2) = \frac{k_{\text{on}}^{(i)} k_{\text{off}}^{(i)} F_i}{\sum_j k_{\text{on}}^{(j)} k_{\text{off}}^{(j)} F_j}. \tag{30}$$

where F_i is the fraction of the initial aptamer library which consists of aptamers of type i. We see the selection favors aptamers which bind to the bead surface both with large binding and unbinding kinetic rates, see equation 30.

A further simplification can be made by considering the idealized case in which the aptamer library has been prepared so that all the aptamers have the same $k_d = k_{\text{off}}/k_{\text{on}}$. In this idealized case, $k_{\text{on}} = k_d^{-1} k_{\text{off}}$. This yields for the fraction of free aptamers in buffer solution

$$Q_i(t_1 + t_2) = \frac{\left(k_{\text{off}}^{(i)}\right)^2 F_i}{\sum_j \left(k_{\text{off}}^{(j)}\right)^2 F_j}. \tag{31}$$

This shows the selection clearly favors aptamers with large k_{off} in the library. The results of selecting aptamers with particular kinetics, as characterized in equations 29 and 30 is shown in Figure 13.

This demonstrates two possible ways in which a microfluidic device could be used to select aptamers not only with small k_d but also with specified kinetic characteristics. It is expected that many other approaches also possible using emmerging microfluidic techniques and processing. The analysis here is meant to give one indication of what may be possible when utilizing such technologies.

IV. CONCLUSIONS

Obtaining atpamers from a molecular library which bind tightly to specific targets molecules still poses many significant challenges. In this work we investigated several features of the selection process and identified possible mechanisms by which selection can be improved. With advances in microfluidic technologies we expect that many new effective approaches may become possible to realize in practice greatly reducing the required laboratory labor and resources required to obtain useful aptamers.

V. ACKNOWLEDGMENTS

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Appendix A: Optimality of Small Target Concentrations in SELEX

We now show that taking the concentration of target molecules as small as practially possible is an optimal strategy in utilizing the standard selection step of the SELEX method. We first show that in approaching the zero target concentration limit the average dissociation constant is always smaller than in the large target concentration limit. We then show that the average dissociation constant obtained from the selection step is a strictly increasing function of the target concentration.

1. Comparison of Average Dissociation Constants for Small and Large Target Concentrations

In the case that both dissociation constants are non-zero, the inequality we seek to show is equivalent to $k_{d,0}^{-1} > k_{d,\infty}^{-1}$. For notational convenience let $p_i = [A_{T,i}]/[A_T]$, $x_i = k_d^{(i)}$, and $f(x) = 1/x$, then

$$k_{d,0}^{-1} = \sum_i p_i f(x_i) \tag{A1}$$

$$k_{d,\infty}^{-1} = f\left(\sum_i p_i x_i\right). \tag{A2}$$

Since $\sum_i p_i = 1$ we have by the strict convexity of $f(x)$ and Jensen's inequality [29, 30] that

$$\sum_i p_i f(x_i) > f\left(\sum_i p_i x_i\right). \tag{A3}$$

This shows that the average dissociation constant obtained during the selection step in the limit of small concentration of target, $[S] \to 0$, will always be smaller than the average dissociation constant obtained in the limit of large target concentration, $[S] \to \infty$. In practice, a regime in between these too extremes will likely be used. We now show that in fact the limit of small concentration of target is globally optimal.

2. Comparison of Average Dissociation Constants for Intermediate Target Concentrations

For notational convenience we shall adopt the conventions above and define $y_i = (k_d^{(i)} + [S])^{-1}$ and $g(y) = y^2$. Differentiating in $[S]$ equation 16 we obtain after some straight-forward algebra the expression

$$g\left(\sum_i p_i y_i\right)\frac{\partial \bar{k}_d}{\partial [S]} = \sum_i p_i g(y_i) - g\left(\sum_i p_i y_i\right).$$

Now since $g(y)$ is strictly convex we have by Jensen's inequality [29, 30] that

$$\sum_i p_i g(y_i) > g\left(\sum_i p_i y_i\right). \tag{A4}$$

Since y_i is non-zero, we have that $\partial \bar{k}_d/\partial [S] > 0$ for all $[S]$.

This shows that the selection step gives an average dissociation constant which is an increasing function of the target concentration level. This shows that in the selection step an improvement in the average dissociation constant obtained can always be achieved by decreasing the amount of available target molecules. The optimal strategy for the selection step is then to take the concentration of target molecules as small as possible. We remark that in practice this result is limited to the physical regime in which mass-action chemical kinetics offers a valid description.

Appendix B: Utilizing Degradation of Aptamers : Analysis of the Linearized Equations

The general solution of the ODE of equation 25 can be expressed as

$$\begin{bmatrix} [A_i](t) \\ [A_{T,i}](t) \end{bmatrix} = c_+^{(i)} e^{\lambda_+^{(i)} t}\mathbf{v}_+^{(i)} + c_-^{(i)} e^{\lambda_-^{(i)} t}\mathbf{v}_-^{(i)} \tag{B1}$$

where $\lambda_\pm^{(i)}$ are the eigenvalues and $\mathbf{v}_\pm^{(i)}$ are the eigenvectors of the linear system on the right-hand-side of equation 25. The eigenvalues are given by

$$\lambda_\pm^{(i)} = -\frac{1}{2}\left(k_{\text{on}}^{(i)}[S_T] + k_{\text{off}}^{(i)} + \gamma\right)\cdot \cdot\left(1 \mp \left(1 - \frac{4k_{\text{off}}^{(i)}\gamma}{(k_{\text{on}}^{(i)}[S_T] + k_{\text{off}}^{(i)} + \gamma)^2}\right)^{1/2}\right). \tag{B2}$$

TABLE I: Description of the Parameters

Parameter	Description
S_T	Total concentration of target Species.
A_T	Total concentration of aptamer Species.
F_i	Initial fraction of the population of aptamter species i.
B_i	Fraction of the population of species i.
$k_d^{(i)}$	Dissociation constant of the aptamer species of type i.
$[k_d^{A\,\min}, k_d^{A\,\max}]$	Thresholds for k_d values defining the aggregate aptamer class labeled A.
$[k_d^{B\,\min}, k_d^{B\,\max}]$	Thresholds for k_d values defining the aggregate aptamer class labeled B.
$[k_d^{C\,\min}, k_d^{C\,\max}]$	Thresholds for k_d values defining the aggregate aptamer class labeled C.

The eigenvectors are given by

$$\mathbf{v}_+^{(i)} = [\lambda_+^{(i)}, -\gamma]^T \tag{B3}$$

$$\mathbf{v}_-^{(i)} = [\lambda_-^{(i)}, -\gamma]^T. \tag{B4}$$

For initial concentrations $[A_i](0)$ and $[A_{T,i}](0)$ the coefficients are given by

$$c_+^{(i)} = \{\gamma[A_i](0) + \lambda_-[A_{T,i}](0)\}/\{\gamma(\lambda_+ - \lambda_-)\} \tag{B5}$$

$$c_-^{(i)} = \{\gamma[A_i](0) - \lambda_+[A_{T,i}](0)\}/\{\gamma(\lambda_+ - \lambda_-)\}. \tag{B6}$$

The fraction bound to the target can then be expressed as

$$B_i(t) = \frac{[A_iS](t)}{[AS](t)} = \frac{p_+^{(i)} e^{\lambda_+^{(i)} t} + p_-^{(i)} e^{\lambda_-^{(i)} t}}{\sum_j p_+^{(j)} e^{\lambda_+^{(j)} t} + p_-^{(j)} e^{\lambda_-^{(j)} t}}. \tag{B7}$$

where $[A_iS](t) = [A_{T,i}](t) - [A_i](t)$ and $p_+^{(i)} = c_+^{(i)}(\mathbf{v}_+^{(2)} - \mathbf{v}_+^{(1)})$ and $p_-^{(i)} = c_-^{(i)}(\mathbf{v}_-^{(2)} - \mathbf{v}_-^{(1)})$. Since $|\lambda_-^{(i)}| > |\lambda_+^{(i)}|$ and $\lambda_\pm^{(i)} < 0$ we have that as $t \to \infty$ the exponential terms corresponding to $\lambda_+^{(i)}$ will dominate for each i. This gives the approximation

$$B_i(t) = \frac{p_+^{(i)} e^{\lambda_+^{(i)} t}}{\sum_j p_+^{(j)} e^{\lambda_+^{(j)} t}}. \tag{B8}$$

In the case there is a unique eigenvalue with minimum magnitude, which we shall index by one $\lambda_+^{(1)}$, the exponential term associated with this eigenvalue will dominate in the denominator of expression B8 as $t \to \infty$. From equation B8 we have $B_1(t) \to 1$ and $B_i(t) \to 0$, $i \geq 2$.

TABLE II: Parameter Values for Studies of Population Levels

Parameter	Value
A_T	1mM.
S_T	0.8mM.
F_i	linear initial profile.
$k_d^{(i)}$	10^{-12}M $- -10^{0}$M.
$[k_d^{A\,\min}, k_d^{A\,\max}]$	$[0, 10^2\text{pM}]$
$[k_d^{B\,\min}, k_d^{B\,\max}]$	$[10^2\text{pM}, 1\mu\text{M}]$
$[k_d^{C\,\min}, k_d^{C\,\max}]$	$[1\mu\text{M}, 1\text{M}]$

TABLE III: Parameter Values for Studies of Purity

Parameter	Value
A_T	1mM.
F_i	linear initial profile.
$k_d^{(i)}$	$10^{-12}\text{M} - -10^{0}\text{M}$.
$[k_d^{A\,\min}, k_d^{A\,\max}]$	$[0, 10^2\text{pM}]$
$[k_d^{B\,\min}, k_d^{B\,\max}]$	$[10^2\text{pM}, 1\mu\text{M}]$
$[k_d^{C\,\min}, k_d^{C\,\max}]$	$[1\mu\text{M}, 1\text{M}]$

TABLE IV: Parameter Values for Studies of Purity with Multiple Target Species

Parameter	Value
A_T	1mM.
$S_T^{(b)}$	10^4mM.
F_i	linear initial profile.
$k_d^{(i)}$	$10^{-12}\text{M} - -10^{0}\text{M}$.
$[k_d^{A\,\min}, k_d^{A\,\max}]$	$[0, 10^2\text{pM}]$
$[k_d^{B\,\min}, k_d^{B\,\max}]$	$[10^2\text{pM}, 1\mu\text{M}]$
$[k_d^{C\,\min}, k_d^{C\,\max}]$	$[1\mu\text{M}, 1\text{M}]$

TABLE V: Description of the Parameters for Degradation Process

Parameter	Description
S_T	Total concentration of target Species.
A_T	Total concentration of aptamer Species.
B_i	Fraction of the population of species i.
$k_{\text{on}}^{(i)}$	Target binding rate constant for the aptamer species of type i.
$k_{\text{off}}^{(i)}$	Target unbinding rate constant for the aptamer species of type i.
γ	Rate of degradation.