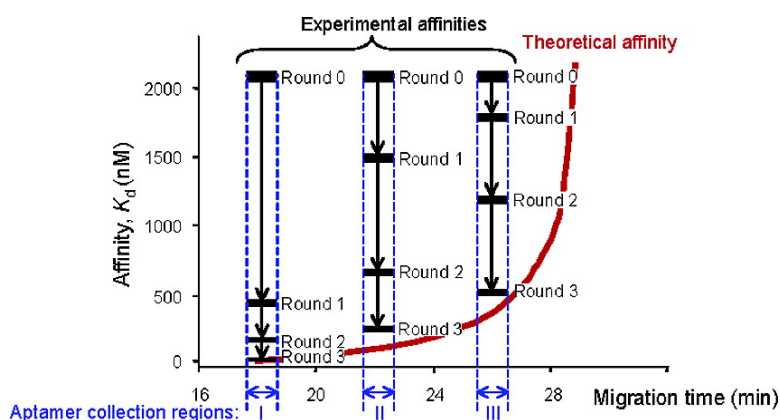


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Selection of Smart Aptamers by Equilibrium Capillary Electrophoresis of Equilibrium Mixtures (ECEEM)

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Aptamers are DNA (or RNA) oligonucleotides capable of binding different classes of targets with high affinity and selectivity.¹ Aptamers are often viewed as artificial antibodies and hold promise as replacements for real antibodies in diagnosis and treatment of diseases.² The designing of advanced aptamer-based diagnostics and therapeutics requires “smart aptamers”—aptamers with pre-defined kinetic and/or thermodynamic parameters of aptamer-target interaction. Technological limitations of aptamer-selection methods have so far precluded selection of smart aptamers. Here, we report for the first time on selection of smart aptamers. We introduce equilibrium capillary electrophoresis of equilibrium mixtures (ECEEM): a method for selection of smart aptamers with pre-defined equilibrium dissociation constants, K_d , of aptamer-target complexes. Conceptually, a mixture of a target with a DNA (RNA) library is prepared and equilibrated. A plug of the equilibrium mixture is injected into a capillary prefilled with a run buffer containing the target at the concentration identical to the target concentration in the equilibrium mixture. The components of the equilibrium mixture are separated by capillary electrophoresis while equilibrium is maintained between the target and aptamers. The unique feature of ECEEM is that aptamers with different K_d values migrate with different and predictable mobilities. Thus, collecting fractions with different mobilities results in smart aptamers with different and predefined K_d values. In this proof-of-principle work, we used ECEEM to select smart aptamers for MutS protein. Three rounds of ECEEM-based selection were sufficient to obtain smart aptamers with K_d values approaching theoretically predicted ones. ECEEM is the first method for aptamer selection whose ability to generate smart aptamers has been experimentally proven. We foresee that other kinetic capillary electrophoresis methods can be designed to select smart aptamers with predefined binding parameters.

A general approach to aptamer selection from libraries of random DNA (RNA) sequences was introduced by Gold's and Szostak's groups in 1990.³ It is called systematic evolution of ligands by exponential enrichment (SELEX) and involves repetitive rounds of affinity partitioning of aptamers from nonaptamers and PCR amplification of aptamers. Bowser's and our groups have recently demonstrated that nonequilibrium capillary electrophoresis of equilibrium mixtures (NECEEM) can be used for highly efficient partitioning of aptamers under nonequilibrium conditions.⁴ The present work instead utilizes the equilibrium approach proposed by Whitesides and coauthors in 1992. In this approach, the quasi-equilibrium between interacting molecules is maintained during capillary electrophoresis (CE) through adding one of the molecules to the run buffer.⁵ The equilibrium approach was initially termed affinity capillary electrophoresis (ACE); later the same term was extended to the whole area of CE-based studies of intermolecular interactions.⁶ To avoid this confusion, we use a more descriptive term of ECEEM instead of ACE.

First, we describe the theoretical bases of ECEEM. By definition, electrophoresis migration time is the time required for a species to

move from the injection end to the detection end of the capillary. We assume that migration times of the target-DNA complex (T -DNA) and free DNA, t_{T-DNA} and t_{DNA} , respectively, are different and known. They can be measured by nonequilibrium capillary electrophoresis of equilibrium mixtures (NECEEM), a process, which is conducted with no target in the run buffer.⁷ We also assume that the DNA library contains aptamers with a variety of K_d values. In ECEEM, the electrophoresis run buffer contains the target at the concentration identical to that in the equilibrium mixture. Due to the dynamic equilibrium between free aptamers and aptamer-target complexes during ECEEM separation, aptamers spend a part of time as complexes with the target, and a part of time as free aptamers. The lower the K_d value, the more time aptamers spend within the complex, and the closer their migration time is to t_{T-DNA} . Vice versa, the higher the K_d value, the more time aptamers spend as free DNA molecules, and the closer their migration time is to t_{DNA} . Due to the dynamic equilibrium, the migration time of aptamers in ECEEM depends on K_d and the concentration of free target, $[T]$, in the following way:

$$\frac{1}{t} = \frac{1}{t_{DNA}} \frac{K_d}{[T] + K_d} + \frac{1}{t_{T-DNA}} \frac{[T]}{[T] + K_d} \quad (1)$$

The concentration of free target can be assumed to be equal to the initial concentration of the target if the target is in excess to aptamers. Rearranging formula 1 leads to the following expression for K_d of selected aptamers as a function of the migration time and target concentration:

$$K_d = [T] \frac{t_{DNA} t - t_{T-DNA}}{t_{T-DNA} t_{DNA} - t} \quad (2)$$

The formula suggests that that if the aptamer-collection window includes the point of $t = t_{T-DNA}$, the lower limit of K_d for selected aptamers is equal to zero. On the other hand, if the aptamer-collection window includes the point of $t = t_{DNA}$, the upper limit of K_d for selected aptamers is equal to infinity. It should be noted that because of the finite widths of electrophoretic peaks and finite widths of aptamer-collection windows, K_d values of selected aptamers are always within a certain finite range of values: $K_d^{\min} < K_d < K_d^{\max}$.

Second, we examined the concept of ECEEM-based selection of smart aptamers with predetermined K_d experimentally. The target used in this work was MutS protein, for which aptamers have never been previously selected. Aptamers were selected from a DNA library with a random sequence of 39 bases. As the first step, we used NECEEM to find t_{T-DNA} and t_{DNA} . Figure 1A shows a NECEEM electropherogram for the mixture of the DNA library with 2.8 μ M concentration of MutS. A small peak with a migration time of 17.8 min corresponds to the complex of MutS with DNA, while a peak with a migration time of 30.1 min corresponds to free DNA. The same NECEEM electropherogram was also used

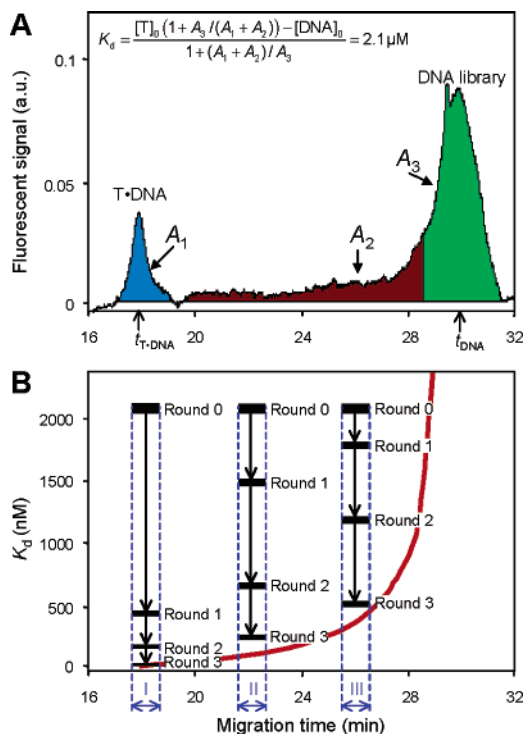


Figure 1. ECEEM-based selection of smart aptamers with predefined K_d for MutS protein as a target. Panel A illustrates determination of migration times, t_{T-DNA} and t_{DNA} , and effective K_d (bulk affinity) of target–library interaction by NECEEM. Concentrations of the target and the library were $2.8 \mu M$ and $1 nM$, respectively. Panel B depicts increasing affinity of the DNA library during ECEEM-based selection of aptamers in three aptamer-collection windows (blue). The red line is the theoretical dependence of the migration time on K_d of target–DNA interaction calculated with expression 2.

to determine bulk affinity (the average K_d value) of the library to be equal to $2.1 \mu M$. NECEEM-based measurements of K_d is described in detail elsewhere.⁷ For selection of smart aptamers by ECEEM we used the concentration of MutS equal to $100 nM$; the initial load of the DNA library was 9×10^{11} molecules. Figure 1B shows the theoretical dependence of K_d on the migration time calculated using expression 2 for $t_{T-DNA} = 17.8 min$, $t_{DNA} = 30.1 min$, and $[T] = 100 nM$. ECEEM fractions were collected within three time windows shown in Figure 1B; the theoretically anticipated K_d values for the three windows lie within three ranges: 0–11, 73–105, and 284–410 nM. Collected DNA was PCR amplified to obtain three enriched libraries. The K_d values for bulk affinity of the enriched libraries to MutS were measured by NECEEM.⁷ Figure 1B illustrates the convergence of experimental K_d values to the theoretically predicted values. The starting point in selection was the bulk affinity of $2.1 \mu M$. The arrows show the progression in K_d with three consecutive rounds of selection. Three rounds of ECEEM-based selection were enough to approach the predicted K_d values. Additional rounds of selection did not significantly change the affinity. We then used bacterial cloning to amplify individual aptamers and used NECEEM to measure K_d values of individual aptamers. They were consistent with the K_d of the three enriched libraries. Figure 2A shows the structure of an aptamer with affinity of $15 nM$, which was obtained from the enriched library I. The red part of the structure represents the random region, and the blue parts represent the constant regions. When the constant regions were truncated, the structure was destroyed (Figure 2B), and the affinity was completely lost ($K_d > 300 \mu M$).

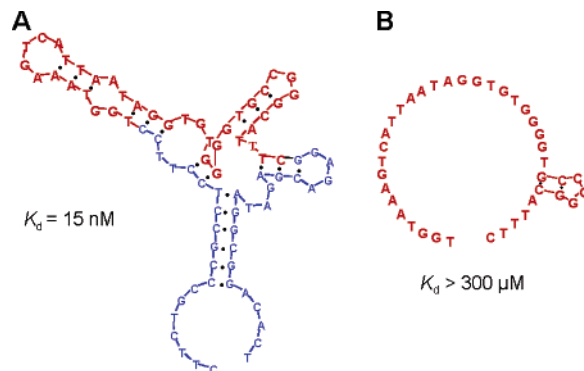


Figure 2. Structures and affinities of an aptamer with (A) and without (B) constant regions (blue).

Finally, we outline the major features of ECEEM. ECEEM is the only method, in which aptamer–target equilibrium is maintained during partitioning of aptamer–target complexes from free DNA. This unique feature of ECEEM is pivotal to its ability to select smart aptamers with predefined values of K_d . Conveniently, K_d values of selected aptamers are dependent on the parameters (migration times and target concentration) in an explicit way (formula 2). To maintain the equilibrium, the method requires that the target be present in the run buffer, which increases target consumption in comparison with aptamer selection by NECEEM.^{4b} However, due to the small diameter of the capillary, target consumption is still much lower than in all methods other than NECEEM and ECEEM. The full power of ECEEM could hardly be realized without NECEEM, which is used to find the migration times, t_{T-DNA} and t_{DNA} , and K_d values.

To conclude, ECEEM represents the first method for selection of smart aptamers with predefined values of equilibrium constants, K_d . Smart aptamers will facilitate designing smarter diagnostics and therapeutics. For example, ECEEM-selected panels of aptamers with K_d values ranging in a wide scope will be used in competitive screening of drug candidates. We foresee that further development of kinetic methods in CE will provide a variety of methodological schemes for high-throughput screening of combinatorial libraries using CE as a universal instrumental platform.

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Supporting Information Available: Supporting materials and methods. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) (a) Famulok, M.; Mayer, G.; Blind, M. *Acc. Chem. Res.* **2000**, *33*, 591–599. (b) Gold, L.; Brody, E.; Heilig, J.; Singer, B. *Chem. Biol.* **2002**, *9*, 1259–1264. (c) Ellington, A. D.; Conrad, R. *Biotechnol. Annu. Rev.* **1995**, *1*, 185–214.
- (2) (a) Rimmel, M. *ChemBioChem* **2003**, *4*, 963–971. (b) Rusconi, C. P.; Scardino, E.; Layzer, J.; Pitoc, G. A.; Ortel, T. L.; Monroe, D.; Sullenger, B. A. *Nature* **2002**, *419*, 90–94. (c) Jayasena, S. D. *Clin. Chem.* **1999**, *5*, 1628–1650.
- (3) (a) Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505–510. (b) Ellington, A. D.; Szostak, J. W. *Nature* **1990**, *346*, 818–822.
- (4) (a) Mendonsa, S. D.; Bowser, M. T. *J. Am. Chem. Soc.* **2004**, *126*, 20–21. (b) Berezovski, M.; Drabovich, A.; Krylova, S. M.; Musheev, M.; Okhonin, V.; Petrov, A.; Krylov, S. N. *J. Am. Chem. Soc.* **2005**, *127*, 3165–3171.
- (5) Chu, Y. H.; Avila, L. Z.; Biebuyck, H. A.; Whitesides, G. M. *J. Med. Chem.* **1992**, *35*, 2915–2917.
- (6) Heegaard, N. H. H.; Nissen, M. H.; Chen, D. D. Y. *Electrophoresis* **2002**, *23*, 815–822.
- (7) Berezovski, M.; Krylov, S. N. *J. Am. Chem. Soc.* **2002**, *124*, 13674–13675.

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