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Development of a Fraction Collection Approach in Capillary Electrophoresis SELEX for Aptamer Selection

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Aptamers have attracted much attention due to their ability to bind to target molecules with high affinity and specificity. The development of an approach capable of efficiently generating aptamers by systematic evolution of ligands by exponential enrichment (SELEX) is particularly challenging. Herein, a fraction collection approach in capillary electrophoresis SELEX (FCE-SELEX) for partition of bound DNA-target complex is developed. By integrating fraction collection with a facile oil seal method for avoiding contamination in amplifying bound DNA-target complex, in a single round of selection, the streptavidin-binding aptamer (SBA) has been generated. The affinity of aptamer SBA-36 for streptavidin (SA) is determined as 30.8 nM by surface plasmon resonance (SPR). Selectivity and biotin competition experiments demonstrate the SBA-36 aptamer selected by FCE-SELEX is as efficient as those from other methods. Based on the ability of fraction collection in partition and collection of aptamer-target complex from original DNA library, FCE-SELEX can be a universal tool for development of aptamers.

Introduction

Aptamers are artificial oligonucleotides (DNA or RNA) capable of binding different classes of targets, such as peptides, proteins, drugs, organic and inorganic molecules or even whole cells, with high affinity and specificity.[1] Aptamers have great potential as analytical, diagnostic and therapeutic tools.[2] Aptamers are normally selected using a process of SELEX, which involves iterative cycles of binding, partition and amplification.[3, 4] Traditional SELEX requires a dozen or more laborious cycles to isolate strongly binding aptamers, which can take months to complete. To shorten the time of selection and adapt SELEX into an appropriate tool for selecting high-affinity ligands, many selection methods have been developed, such as nitrocellulose filter binding SELEX,[5] bead-based SELEX,[6, 7]microfluidic SELEX,[8] and capillary electrophoresis-SELEX (CE-SELEX).[9] Among them, capillary electrophoresis (CE) has been reported to be a very efficient method for separation of bound and unbound DNA/RNA.[9-15] Basing on CE's trait, non-equilibrium capillary electrophoresis of equilibrium mixtures (NECEEM) and equilibrium capillary electrophoresis of equilibrium mixtures(ECEEM) had been developed as universal method for development of aptamers and accurately determined binding parameters (K_d, k_{on}, and k_{off}) of the aptamer-target interaction.[11, 12] However, due to the scarce target-DNA complex in the primary selection, after electrophoretic migration no peaks of target-DNA complexes were detectable with UV absorbance detection or even with fluorescence detection. The undetectable target-DNA complexes were only collected blindly within the wide aptamer collection window, and then all collected DNA was amplified in one tube using polymerase chain reaction (PCR).[11, 14] consequently, the high affinity aptamers mixed together with other weak affinity DNA, or even free DNA. Thus more rounds may need to generate aptamer. Recently, Dovichi group developed a fraction collector for CE automated fraction collection to collect different time window into many separated well.[16] However, the great limitation of their method is sample will be subjected to over 1000-fold dilution during fraction generation. Thus, the samples are easy loss, contamination, and difficult to amplify. Therefore, it is imperative to develop a facile approach for achievement of efficient isolation and collection the bounded target-aptamer complex.

After primary isolation and collection of the target-aptamer complex, amplification is necessary for productive SELEX process. PCR is the most widely used method to amplify aptamer and generate the enriched library. Compared to the development of separation approaches, only few PCR amplification strategies in this field have been reported.[17, 18] Amplification and generation of enriched libraries can be problematic by PCR, such as, generation of by-products, PCR

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bias, and contamination.[19] To reduce by-product formation, emulsion PCR was used in SELEX experiments for amplification.[20, 21] In PCR, for a random DNA library as a template, product accumulation stop when PCR primers are still in excess of the products. Therefore, optimizing the cycles of PCR could reduce by-products.[22] If the heterogeneity of template sequences with different GC contents, non-uniform amplification during the PCR because of secondary structure formation would appear. This non-uniform amplification, then, biases the composition of DNA sequences in the amplified library and, consequently, would affect the sequences selected for aptamers. Sometimes, sequences forming stable secondary structures lost due to PCR bias were just aptamers for target.[23, 24] Therefore, Gorenstein group developed a smart way to reduce PCR bias by combination of 1 M betaine and 5% DMSO in PCR amplification.[17] Except for by-product and PCR bias, PCR contamination is another annoying, but common problem. A laboratory, in which different targets aptamer selections using different DNA libraries and methods have been carried out, is full of DNA aerosol in the air or on the machines, pipettes even lab coats. These unexpected and unsolicited contaminations would affect the aptamer amplification. However, little solution has been reported for solving the PCR contamination issue to date.

This work is presented to explore a fraction collection approach in CE-SELEX (named FCE-SELEX) for aptamer selection - a process, which involves partition of the aptamer collection window into many sections and collection of every section time window into a separate PCR tube which is preloaded with the oil-sealed PCR mixture. Subsequently, amplification of every tube is performed by real time PCR. The preloaded and oil-sealed method, according to we survey, has not yet been brought in CE-SELEX. Thus the limited aptamer coming from fraction collection can be immediately amplified and also be avoided any unsolicited contamination. The enriched DNA library with a certain level of bulk affinity is defined as the DNA from the tube with the minimum quantification cycle (C_q) at half of maximum fluorescence in real time PCR and lower K_d value in CE, after that individual DNA molecules from the enriched library are selected by bacterial cloning, sequenced, and chemically synthesized. Uniquely, a single round of fraction-collection selection and PCR amplification is sufficient for obtaining the high affinity aptamers.

Experimental

DNA library and primers

The DNA library contains a central randomized sequence of 40 nucleotides flanked by 20-nt primer hybridization sites. A 6-carboxyfluorescein-labelled 5'-primer and another primer contains hexaethylene glycol and an extension of 20-nucleotides length of a string of adenosine were used in PCR reactions for the synthesis of labelled double-stranded DNA molecules. DNA Library and primers were synthesized by a company (Sangon, Shanghai, China). The library (1.3 nmol) was dissolved in 13 μL of water to get 100 μM stock solutions and stored at -20 °C. The predicted secondary structures of aptamers were generated by Mfold software.

Capillary electrophoresis

All CE procedures were performed using the following instrumentation and common settings and operations unless otherwise stated. CE was carried out with a PA800 plus apparatus (Beckman Coulter, USA) equipped with absorption and fluorescence detectors; a 488 nm line of an Ar ion laser was utilized to excite fluorescence. A 50 cm long (40 cm to a detection window) uncoated fused silica capillary with an inner diameter of 75 μm and outer diameter of 360 μm was used. Both the inlet and the outlet reservoirs contained the electrophoresis run buffers 50 mM sodium tetraborate at pH 8.4. The samples were injected into the capillary, prefilled with the run buffer, by a pressure pulse of 5s × 0.5psi (3.35 kPa). Electrophoresis was carried out for a total of 20 min by an electric field of 375 V/cm with a positive electrode at the injection end of the capillary; the direction of the electroosmotic flow was from the inlet to the outlet reservoir. The temperature of the separation capillary was maintained at 25 ± 0.1 °C. When needed, fractions were collected in an automated mode by replacing the regular outlet reservoir with a fraction collection tube containing 50μL of PCR mixture. The capillary was rinsed with the run buffer solution for 2 min prior to each run. At the end of each run, the capillary was rinsed with 100 mM NaOH for 2 min, followed by a rinse with deionized water for 2 min, running buffer wash 2 min(20psi).

Selection of aptamers

First, 5 μL of the 20 μM DNA library solution in the selection buffer (50 mM sodium tetraborate 5 mM $MgCl_2$ at pH 8.4) was denatured by heating at 95 °C for 10 min with subsequent cooling to 20 °C . Second, 5 μL of the 25 nM SA solution in the selection buffer was mixed with the DNA library sample and incubated at 25 °C for 30 min. A 25 mm long (110 nL) plug of the equilibrium mixture was injected into the capillary prefilled with the run buffer; The injected equilibrium mixture was subjected to non-equilibrium capillary electrophoresis at an electric field of 375 V/cm and with the temperature of the capillary at 20± 0.1 °C. 8 min after the beginning of non-equilibrium capillary electrophoresis of equilibrium mixtures (NECEEM), the regular outlet reservoir was replaced with the fraction collection tube. Every fraction collection PCR tube contains 50 μL of PCR mixture (50 mM KCl, 10 mM Tris- HCl (pH 8.6), 2.5 mM $MgCl_2$, all four dNTPs at 200 μM each, primers (0.3 μM each), and 0.05 unit/μL Taq DNA polymerase) and is sealed by mineral oil (sigma, M3516). For the following 6 min, electrophoresis was collected into different PCR tubes (0.5min/fraction), and then electrophoresis was run for an additional 4 min, the fraction collection tubes was replaced with a collection vial for free DNA library.

DNA collected into the PCR tubes was fluorescent quantitative PCR amplified in Light Cycler 96 System (Roche, Switzerland). 30 cycles were conducted, with every cycle consisting of

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melting at 94 °C for 30 s, annealing at 60 °C for 15 s, and extension at 72 °C for 15 s. The 6-carboxyfluorescein-labelled ssDNA was separated from the complementary ssDNA strand by size separations on denaturing urea polyacrylamide gel electrophoresis.[25]

The enriched DNA library was cloned into *E. Coli.* using the pT7 Blue-3 Perfectly Blunt Cloning Kit (Novagen, Madison, WI). Colonies grown on agar plates were picked randomly for sequencing of DNA. A plasmid from each colony was prepared using a GenElute Plasmid Miniprep Kit and 40 clones were sequenced by Sangon, (Shanghai, China).

Determination of the dissociation constant (K_d)

The affinity of the selected aptamers for SA was determined by Biacore assay as before we used.[26] In brief, a capturing streptavidin (Amresco, Solon, OH, USA) was immobilized on a CM5 dextran sensor chip (Pharmacia Biosenso, Piscataway, NJ, USA) in 10 mM sodium acetate (pH 4.5) for 7 min using the Amine Coupling kit (Biacore). The dextran layer of the sensor chip was activated or deactivated by injection of 35 µl of N-ethyl-N0-(3-dimethyllaminopropyl) carbodiimide or N-hydroxysuccinimide, respectively, for 7 min. Excessive reactive groups were blocked by the injection of 35 µl of 1 M ethanolamine, pH 8.5. Next, 5 µM of the selected aptamers in Tris-HCl buffer (20 mM, 2mM EDTA, pH 8.0) was injected, and the analyses were conducted at a flow rate of 1 µl/min at 25 ± 0.1 °C. The surface was regenerated with 10 mM sodium-glycine-HCl (pH 2.25) at a flow rate of 5 µl/min. K_d was evaluated using BIA evaluation 4.0 software supplied by the manufacturer (Biacore). Other protein (BSA, HSA, huam-SOD1 and trypsin purchased from Sigma) replaced SA to immobilize on CM 5 chips for the selectivity experiments. Other conditions are the same as SA.

Biotin competitive assays

A total of 100 µg of streptavidin-coated magnetic beads were incubated with 50 pmol of the FAM-labelled ssDNA aptamers in 500 µl Tris-HCl buffer (20 mM, 2mM EDTA, pH 8.0) at 25 ºC for 1 h. After the binding reaction, the beads were washed five times with 500 µl binding buffer to remove unbound aptamers. Non-aptamers incubated with streptavidin-coated magnetic beads was used as a blank control. The washed streptavidin coated beads containing bound fluorescein-labeled aptamer mixtures were re-suspended in 200 µl binding buffer and incubated with different amount of biotion(0-80 fmol) at 25ºC for 1 h. After the competitive reaction, the beads were washed three times with 500 µl of binding buffer to remove unbound aptamers and biotion. The FAM fluorescence of streptavidin-coated magnetic beads was monitored with a FACSCalibur cytometer (Becton, Dickinson and Company, San Jose, CA, USA).

Results and discussion

Concept of NECEEM-based fraction collection

Non-equilibrium capillary electrophoresis of equilibrium mixtures (NECEEM) has been demonstrated as a universal tool for development of aptamers and accurately determine binding parameters (K_d, k_{on}, and k_{off}) of the aptamer-target interaction.[11] Ideally, in the electric field, the zones of free DNA, DNA-target complex (DNA-T), and target (T) are separated. The lower the K_d value, the more time aptamers spend within the target, and the closer their migration time is to T_T. 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}.[12] In practice, take our particular protein target as example, the mobility of DNA-Protein will typically be intermediate between those of DNA and protein, that is a whole electropherogram components in order: (1)free protein; (2) specific DNA-protein complex (with lower K_d value); (3) non-specific DNA-protein complex (with higher K_d value); (4) free DNA. As illustrated in scheme 1, to ensure no aptamers missing, the aptamer collection window is chosen to span from the first protein component to the close proximity of the original library. Our fraction collection method involves in partition the blindly wide aptamer collection window into many iso-time fractions, which are determined according to the half peak width (HPW) of protein and DNA library, and then respectively collects every sub-time window sample into a separate PCR tube, which is preloaded with PCR mixture. Furthermore, the PCR mixture is oil sealed to isolate from air, thus avoiding the contamination from environmental DNA aerosol. Thereby, the rare protein-DNA complex could be effectively amplified. Oil seal method was used to avoid the evaporation of PCR mixture in the primary development of PCR. Here, oil seal is used as a method to overcome the PCR contamination. The oil is placed the top of the PCR mixture resulting in the isolation of the air but not affect the PCR reaction. Ultimately, integrating fragment collection method with preloading and oil seal strategy, we restrain from the blindness and non-specificity collection in traditional CE-SELEX and improve the efficiency of amplification. Therefore, FCE-SELEX can be adapted as an appropriate tool for selecting high-affinity ligands.

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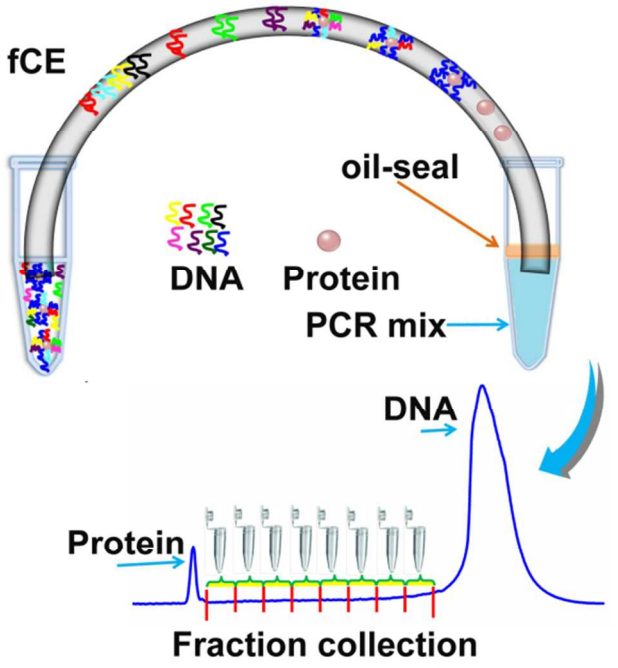

Scheme 1. Illustration of FCE-SELEX selection for aptamers.

Single round selection of aptamers for streptavidin (SA)

Subsequent experiments were aimed at demonstrating FCE-SELEX approach by taking streptavidin as a typical example for apatmer selection. Streptavidinis a homo-tetramers with an extraordinarily high affinity for biotin (dissociation constant (K_d) on the order of ~10^{-14} M).[26] Based-on the strongest non-covalent interaction; streptavidin is widely used in bio-analysis and purification. However, the difficulty to separate biotinylated molecules from streptavidin to some extent limits their applications. Since aptamers possess appropriate binding ability and great flexibility in probe/sensor design, streptavidin-binding aptamer(SBA) can be a good alternative of biotin for developing the streptavidin-based technology. In addition, SA would be a better defined model target since SBAs had been selected by different laboratories using different methods. Thus, the efficiency of different selection approaches can be easily compared.[26-29] Aptamers were selected from a DNA library with a random sequence of 40 bases flanked by two constant primer regions of 20 and 20 bases (overall 80 bases, 25 kDa, table S1). The 5' end of the library was fluorescently labelled with 6-carboxyfluorescein (FAM). Electrophoresis was carried out at an electric field of 375 V/cm in a 50 cm long capillary with an inner diameter of 75 μm.

Table 1Sequences and affinities of aptamers

Name	Center sequence*	Affinity (nM)
SBA-36	GGACGCACCGATCGCAGGTTCCGATA TGACTACTTGGTTG	30.8±0.5
SBA-8	TGACGCACCGATCGCAGGTTCCGATA TGACTACTTGGTTA	149±0.8
SBA-16	TGACGCACCGATCGCAGGTTCCGATA TGACTACTTGGTTG	157±0.5
SBA-19	_GACGCACCGATCGCAGGTTCCGAAA TGACTACTTGGTTG	244±0.3

*5'-AGCAGCACAGAGGTCAGATG-(center)-CCTATGCGTGCTACCGTGAA -3', the full length sequences include two primer hybridization sites and the center random sequence.

First, we defined the aptamer collection window for partitioning by NECEEM. As shown in Figure 1a and b, the migration time of SA and DNA library peaked at 6.9 min and 16 min respectively. The next step was NECEEM-based partitioning of SA-DNA complexes from free DNA. We targeted selection of aptamers with nanomolar affinity; therefore, the equilibrium mixture used for partitioning contained 25 nM SA. As we expected, no peaks of SA-DNA complexes were detectable with UV absorbance detection (Figure 1c) or even with fluorescence detection. Undetectable SA-DNA complexes were collected from the SA protein component to the close proximity of the DNA library. To ensure the efficiency of partitioning and no aptamers missing, we divided collection window from 8.0 min to 14 min into 12 fragments (0.5min/fragment) and collected them into 12 PCR tubes in which PCR mixture was preloaded and oil-sealed; and then the collected DNA was immediately amplified using real time quantitative PCR with a fluorescently labelled forward primer, and a polyA-labeled reverse primer. Fraction collection has been widely used in chromatography as well as in CE,[16, 30-32] yet it is not well used in aptamer development due to the difficulty to determine the fraction time of undetectable aptamer-target complex. The shorter the collection time is the more homogeneous for sample, but shorter time determines fewer samples in one collection resulting in difficulty to amplify; wider collection will cause the high affinity aptamers mixing together with weak affinity DNA, or even free DNA, resulting in fraction collection meaningless. The fraction time used in this study is experiential but referring to HPW of protein and DNA library. In this study, the HPWs of 1μM SA and 10 μM DNA library are 0.5 min and 1.2 min, respectively (Figure 1a and b). To effectively isolate and collect, the fraction time was chose as 0.5 min per section (as shown in Figure 1c). Subsequently, the fragment directly collected into an oil-sealed PCR mixture, thereby reducing the chance of contamination in preparation of amplification. The eighth tube was the primary detectable DNA in real time PCR with C_q value at 18 cycles and followed by the third tube with C_q value at 19 cycles (Figure 2 and Figure S1). Yet, theoretically, the K_d value of complex from the third tube was lower than that from the eighth tube.[33] According to less C_q and lower K_d value principle, DNA from the third tube was defined as the enriched library.

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Other collections, with weak affinity SA-DNA complexes or even free DNA were too less to amplify and analyse by real time PCR.

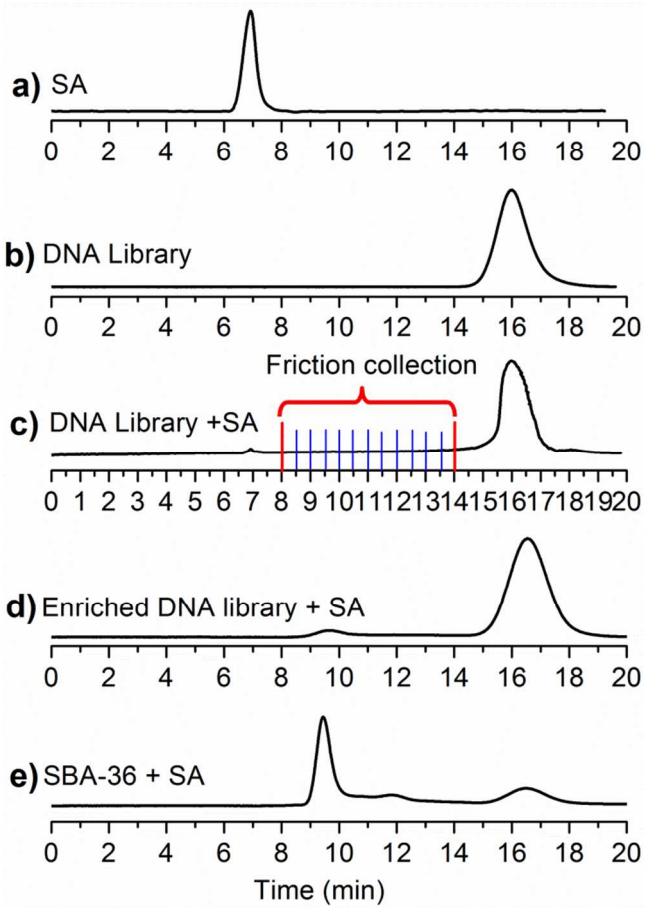

Figure 1. FCE-SELEX selection of aptamers for SA. (a) Electrophoretic migration of SA (1μM); y-axis of the electropherogram represents the absorbance at 280 nm. (b) Electrophoretic migration of DNA library (10μM); y-axis of the electropherogram represents the absorbance at 260 nm. (c) NECEEM-based partitioning of aptamers for SA. The equilibrium mixture contained 10μM DNA library and 25 nM SA; y-axis of the electropherogram represents the absorbance at 260 nm. (d) NECEEM-based determination of the affinity of the enriched DNA library for SA. The equilibrium mixture contained approximately 10 nM fluorescently labelled enriched DNA library and 50 nM SA. y-axis of the electropherogram represents the fluorescence, λ_{ex}: 488 nm. (e) NECEEM-based determination of the affinity of single stranded DNA (ssDNA) molecule cloned from the enriched DNA library for SA. The equilibrium mixture contained approximately 10 nM fluorescently labelled DNA and 100 nM SA, y-axis of the electropherogram represents the fluorescence, λ_{ex}: 488 nm.

Next, ssDNA strands from enriched library (the third tube after amplification by PCR) were separated using size separations on denaturing urea polyacrylamide gel electrophoresis.[25] The reverse primer contained hexaethylene glycol and an extension of 20-nucleotides length of a string of adenosine (table S1). The forward primer was labelled by FAM; these modifications impeded positive strand elongation and created a size difference of the amplicon strands, respectively. The resulting strands of different sizes were subsequently separated on denaturing urea-PAGE. The differential migration enabled selective excision of the strand of ssDNA with the aid of fluorescence. The fluorescently labelled ssDNA were collected to establish the affinity of enriched DNA library. We then prepared the equilibrium mixture of the enriched DNA library with SA and subjected it to NECEEM. A peak corresponding to SA-DNA complexes was observed in a time window of 8.6-10.6 min with peak at 9.6 min, which was an approximate time to the third tube implied in collection window (Figure 1d).

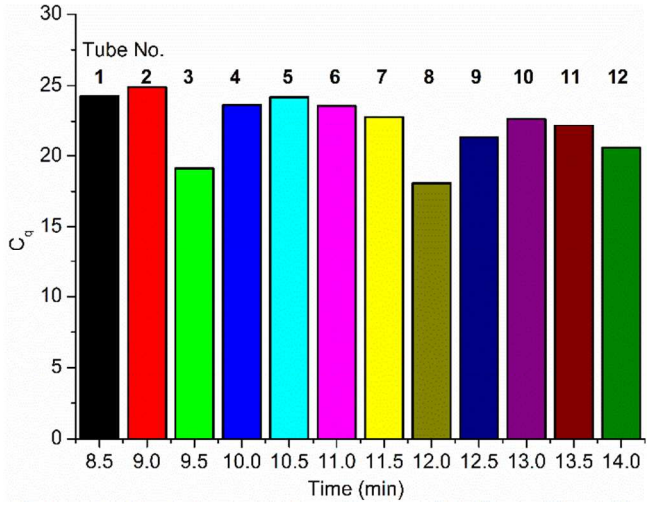

Figure 2. Real time PCR analyses 12 sections of fraction collection, Time = tube No. × 0.5 + 8.

Subsequently, we cloned individual DNA molecules from the enriched library in *E. coli.*and PCR-amplified them with a fluorescent primer. Individual DNA molecules were screened by NECEEM for binding to SA. Approximately 75% of them revealed high affinity and NECEEM electropherograms were qualitatively similar to those of the enriched library (Figure 1d and e). Sequences of the best binders were determined, synthesized and fluorescently labelled (Table 1). The secondary structures of the four aptamers have melting temperatures in a range of 32-42 °C. The affinity of the aptamer SBA-36 was determined as 30.8±0.5 nM by SPR assay (Table 1 and Figure 3). Comparing with the former SA aptamers reported by us in 2009,[26] The K_d (30.8nM) value of SBA-36-SA complex through FCE-SELEX method is lower than that (69.3 nM) using magnetic bead-based SELEX process. FCE-SELEX takes hours to generate SA apatmer enriched library from original pool. It displays unprecedented higher efficiency than former methods.[26-29] Bing et al. compared the secondary structures of all SA aptamers and found a conservative bulge-hairpin structure existence in streptavidin-binding aptamers even them selected by different laboratories using different methods.[29] As shown in the Figure S2, all the four sequences by FCE-SELEX have the conservative bulge-hairpin structure.

To examine the selectivity of our aptamer, we studied the binding to other proteins (HAS, BSA, Tyrisin and human Cu, Zn-SOD); no cross-reactivity was observed (Figure 3). Furthermore, we evaluated the influence of the aptamer on SA-biotin interaction when aptamer SBA-36 was mixed with SA prior to the binding of biotin, the fluorescence intensity

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resulting from aptamer binding was decreased by the addition of biotin in a concentration-dependent manner (Figure. 4). All results demonstrated that aptamer SBA-36 selected by FCE-SELEX is as efficient as those from other methods.

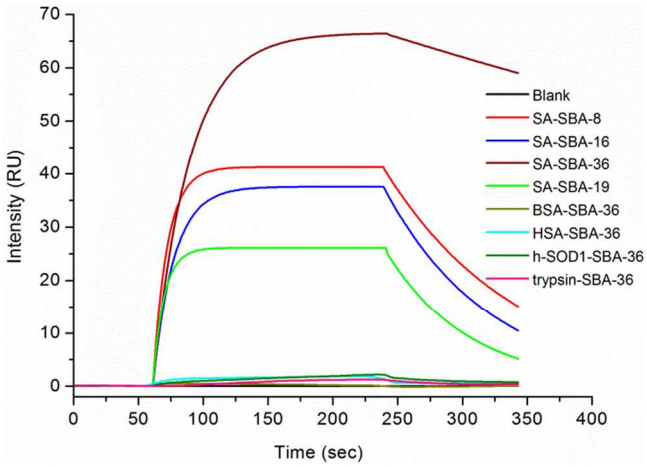

Figure 3. Affinity analysis of aptamers interaction with SA by SPR. The concentration of the selected aptamers was 0.5 μM. Data are presented as real-time graphs of response units (RU) against time and were evaluated using BIAevaluation 4.0 software (Biacore).

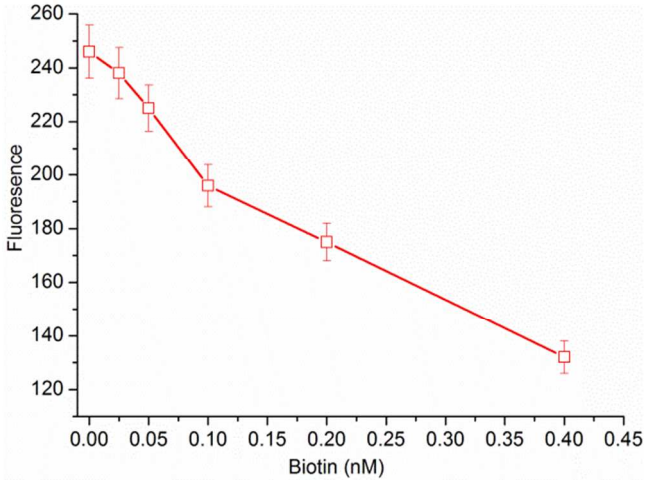

Figure 4. Effects of biotin (0- 0.4 nM) on the binding of aptamer SBA-36 to streptavidin-coated magnetic beads. The assay was evaluated by flow cytometry. Values are represented as means (n = 3).

Conclusions

In summary, FCE-SELEX has several features in selection of aptamers. The first advantage is its speed and simplicity. In this study, for the SA case, the enriched library generating from original DNA library takes a few hours by FCE-SELEX, and fraction collection can be performed in an automated fashion using a commercially available capillary electrophoresis instrument. The second advantage of FCE-SELEX is the collector fabricating with the preloaded and oil-sealed PCR mixture tube. Immediate amplification and contaminative avoidance ensure the productive SELEX. The third and most remarkable advantage of FCE-SELEX is it makes the selection of aptamer in a real single round. Affinity, selectivity and biotin competition experiments reveal that the SBA-36 aptamer is an efficient ligand to bind SA. All results demonstrate that FCE-SELEX can be a high-performance aptamer selection approach.

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Notes and references

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†Electronic Supplementary Information (ESI) available: sequence of primers and library; real time PCR data; the predicted secondary structures of SA apatmers. See DOI: 10.1039/b000000x/

1 M. Famulok, G. Mayer and M. Blind, *Acc. Chem. Res*, 2000, **33**, 591-599.
2 Y. Yang, D. Yang, H. J. Schluesener and Z. Zhang, *Biomol. Eng.*, 2007, **24**, 583-592.
3 C. Tuerk and L. Gold, *Science*, 1990, **249**, 505-510.
4 A. D. Ellington and J. W. Szostak, *Nature*, 1992, **355**, 850-852.
5 I. Wong and T. M. Lohman, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, **90**, 5428-5432.
6 M. A. Lochrie, S. Waugh, D. G. Pratt, J. Clever, T. G. Parslow and B. Polisky, *Nucleic Acids Res.*, 1997, **25**, 2902-2910.
7 X. Lou, J. Qian, Y. Xiao, L. Viel, A. E. Gerdon, E. T. Lagally, P. Atzberger, T. M. Tarasow, A. J. Heeger and H. T. Soh, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 2989-2994.
8 G. Hybarger, J. Bynum, R. F. Williams, J. J. Valdes and J. P. Chambers, *Anal. Bioanal. Chem.*, 2006, **384**, 191-198.
9 S. D. Mendonsa and M. T. Bowser, *J. Am. Chem. Soc.*, 2003, **126**, 20-21.
10 S. D. Mendonsa and M. T. Bowser, *Anal. Chem.*, 2004, **76**, 5387-5392.
11 M. Berezovski, A. Drabovich, S. M. Krylova, M. Musheev, V. Okhonin, A. Petrov and S. N. Krylov, *J. Am. Chem. Soc.*, 2005, **127**, 3165-3171.
12 A. Drabovich, M. Berezovski and S. N. Krylov, *J. Am. Chem. Soc.*, 2005, **127**, 11224-11225.
13 S. D. Mendonsa and M. T. Bowser, *J. Am. Chem. Soc.*, 2005, **127**, 9382-9383.
14 M. Berezovski, M. Musheev, A. Drabovich and S. N. Krylov, *J. Am. Chem. Soc.*, 2006, **128**, 1410-1411.
15 C. Schou and N. H. H. Heegaard, *Electrophoresis*, 2006, **27**, 44-59.
16 Bonnie Jaskowski Huge, Ryan J. Flaherty, Oluwatosin O.Dada and N. J.Dovichi, *Talanta*, 2014, **130**, 288-293.
17 J. Kang, M. S. Lee and D. G. Gorenstein, *J. Biochem. Biophys. Methods*, 2005, **64**, 147-151.
18 K. Shao, W. Ding, F. Wang, H. Li, D. Ma and H. Wang, *Plos One*, 2011, **6**.
19 A. Ozer, J. M. Pagano and J. T. Lis, *New Technologies Provide Quantum Changes in the Scale, Speed, and Success of SELEX Methods and Aptamer Characterization*, 2014.
20 R. Williams, S. G. Peisajovich, O. J. Miller, S. Magdassi, D. S. Tawfik and A. D. Griffiths, *Nat. Methods*, 2006, **3**, 545-550.
21 T. Schuetze, F. Rubelt, J. Repkow, N. Greiner, V. A. Erdmann, H. Lehrach, Z. Konthur and J. Gloekler, *Anal. Biochem.*, 2011, **410**, 155-157.
22 M. U. Musheev and S. N. Krylov, *Anal. Chim. Acta*, 2006, **564**, 91-96.
23 F. Pileur, M. L. Andreola, E. Dausse, J. Michel, S. Moreau, H. Yamada, S. A. Gaidamakov, R. J. Crouch, J. J. Toulme and C. Cazenave, *Nucleic Acids Res.*, 2003, **31**, 5776-5788.

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24 G. Ghosh, D. B. Huang and T. Huxford, *Curr. Opin. Struct. Biol.*, 2004, **14**, 21-27.
25 K. P. Williams and D. P. Bartel, *Nucleic Acids Res.*, 1995, **23**, 4220-4221.
26 C. Wang, G. Yang, Z. Luo and H. Ding, *Acta Biochim. Biophys. Sin.*, 2009, **41**, 335-340.
27 J. A. Bittker, B. V. Le and D. R. Liu, *Nat. Biotech*, 2002, **20**, 1024-1029.
28 R. Stoltenburg, C. Reinemann and B. Strehlitz, *Anal. Bioanal. Chem.*, 2005, **383**, 83-91.
29 T. Bing, X. Yang, H. Mei, Z. Cao and D. Shangguan, *Bioorg. Med. Chem.*, 2010, **18**, 1798-1805.
30 Q. L. Xie, K. E. Markides and M. L. Lee, *J. Chromatogr. Sci.*, 1989, **27**, 365-370.
31 K. D. Altria and Y. K. Dave, *J. Chromatogr.*, 1993, **633**, 221-225.
32 M. Minarik, F. Foret and B. L. Karger, *Electrophoresis*, 2000, **21**, 247-254.
33 M. Berezovski, R. Nutiu, Y. F. Li and S. N. Krylov, *Anal. Chem.*, 2003, **75**, 1382-1386.

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