A systematic evolution of ligands by exponential enrichment workflow with consolidated counterselection to efficiently isolate high-affinity aptamers

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Efficient selection of nucleic acid aptamers with high affinity and specificity for a broad range of targets remains challenging. Historically, aptamer selections have been protracted and tedious processes, often requiring double-digit rounds of selection to converge nucleic acid pools into a small number of prospective high-affinity aptamers. More recently, the use of microfluidic devices and specialized equipment has helped streamline the aptamer selection process, but these platforms are not necessarily accessible to the broad research community. Here, we demonstrate that aptamers with high affinity and moderate specificity can be obtained with a conventional selection workflow that is modified to include facile methods for increasing partitioning and enhancing selection stringency. This process exposes an immobilized protein target to a single-stranded DNA library, followed by washing with buffer that contains the undesired off-target(s), with both steps occurring under constant perfusion using a standard peristaltic pump. Prospective aptamers are then eluted, amplified by an emulsion polymerase chain reaction, regenerated to single strands by enzymatic digestion, and resubjected to the selection procedure. We validated this selection scheme using the platelet-derived growth factor (PDGF) family, whereby we successfully isolated nanomolar affinity aptamers against PDGF-BB with specificity comparable to an aptamer selected using a microfluidics-based approach.

**KEYWORDS**
aptamer, DNA, emulsion polymerase chain reaction, high-throughput sequencing, systematic evolution of ligands by exponential enrichment

1 | INTRODUCTION

Aptamers are single-stranded DNA (ssDNA) or RNA molecules that individually form unique tertiary structures that can bind to a variety of targets, including proteins, small molecules, inorganic compounds, and cell membrane receptors. Since their initial discovery, research interest in aptamers for therapeutic and diagnostic applications has increased primarily due to the potential benefits of aptamers relative to other affinity reagents, including, but not limited to, their smaller size, low immunogenicity, high stability, ease of chemical modifications, and ability to be produced in vitro. In
addition, aptamers offer the chemical flexibility of small molecules while retaining the strong specific binding of antibodies. However, despite their apparent promise, aptamers lag behind other affinity reagents in terms of widespread use.

One oft-cited roadblock for the development of aptamers is the numerous issues associated with the selection workflow. Aptamers are isolated from random or partially structured nucleic acid libraries through iterative rounds of selection in a process termed systematic evolution of ligands by exponential enrichment (SELEX). In a typical round of selection, a positive incubation step is first employed, where the aptamer library is incubated with a primary target molecule. Next, the nucleic acids are subjected to a partitioning step, whereby weakly bound aptamers are removed from the selection pool through washing, amplified by polymerase chain reaction (PCR), and regenerated from double-stranded DNA (dsDNA) into single strands for the next round of selection. To impart specificity into a selection workflow, the library is exposed to undesired or similarly structured secondary targets to remove nonspecific aptamers. This step typically occurs in separate selection rounds by collecting the aptamers that do not bind to the immobilized off-targets. When an aptamer pool has converged through iterative rounds, individual sequences are identified by Sanger sequencing and subsequently characterized for their affinity and specificity to the targets and off-targets. Historically, conventional SELEX has required double-digit selection rounds to converge a library, such that when the final pool is cloned into a plasmid and analyzed by Sanger sequencing, the resulting hits are likely to be high-quality aptamers rather than contaminating nucleic acid strands. More recently, to bypass this need for nucleic acid pool convergence, high-throughput sequencing (HTS) has improved the ability of researchers to identify high-affinity aptamers more rapidly. This method is particularly powerful because the most abundant and/or most enriched sequences can be tracked in early rounds of selection, and these sequences typically have high affinity for the target of interest.

Within the overall SELEX process, two particular challenges that influence aptamer isolation are poor partitioning during selection and washing, which is critical for removing weakly bound aptamers from the selection, and spurious PCR amplification, which can lead to the formation of byproducts and amplification artifacts. Increasing the total number of rounds counteracts these issues and improves the chances of isolating high-affinity aptamers, but increases the workload and overall time required to obtain useful aptamers. To address these issues and shorten the SELEX workflow, several research groups have developed novel strategies and/or devices. For example, to overcome poor partitioning, more stringent washing steps have been incorporated into remove weakly bound aptamers within microfluidic devices. Another method, capillary electrophoresis, does not require target immobilization and uses low reaction volumes. Improved buffers, passivated surfaces, and removal of binding interference by the fixed primer regions in the nucleic acid library have also been employed to enhance partitioning. In addition, to reduce the chance of byproduct formation during the amplification step, droplet digital PCR (ddPCR), which employs emulsion PCR principles to compartmentalize individual nucleic acid sequences, has been incorporated into selections. However, despite these advancements, several concerns remain with respect to SELEX workflows. First, the necessary devices, equipment, and/or technical expertise described above (eg, microfluidic cartridges, ddPCR machines, and capillary electrophoresis setups/expertise) are not always readily accessible to the broader research community. Second, as previously mentioned, the canonical route for imparting selectivity during SELEX is through additional rounds of negative selection against off-target molecules, which increases the amount of labor and reagent costs required to perform successful selections. As such, in this work, we sought to address these issues to more broadly enable the isolation of aptamers. We explored whether certain principles from advanced SELEX workflows, particularly the use of dynamic flow conditions during selection and washing and compartmentalized PCR techniques during sequence recovery and amplification, could be incorporated into more traditional platforms. We also explored an expedited approach to counterselection, whereby off-targets were included during the washing stage, thus establishing an environment for competitive binding of prospective aptamers between the immobilized target and soluble off-targets while eliminating the need for a separate counterselection step. Using the platelet-derived growth factor (PDGF) family as a model system, we demonstrate that this integrated SELEX workflow is able to isolate aptamers to the primary target (PDGF-BB) with low nanomolar affinity and moderate specificity relative to other PDGF homodimers. Ultimately, these aptamers were identified without the need for a microfluidics-based selection or uncommon PCR equipment, and the highest quality aptamers are comparable to ones selected against PDGF-BB using microfluidics. Because all of the materials and assays used in this workflow are easily accessible to researchers, we suggest that the techniques described herein may be broadly adapted for performing aptamer selections.
2 | MATERIALS AND METHODS

2.1 | Materials and instruments

Recombinant human PDGF-AA, -BB, and -CC were purchased from Peprotech (Rocky Hill, NJ) and recombinant human PDGF-DD was purchased from R&D Systems (Minneapolis, MN). All chemicals were purchased from Fisher Scientific (Hampton, NH). The starting DNA library, primers, and custom aptamer sequences were purchased from Integrated DNA Technologies (San Jose, CA) with HPLC purification. The starting DNA library was synthesized with 40 nucleotide random bases flanked by 20 nucleotide primer ends required to perform PCR amplification (forward fixed region: TCGCACATTCCGCTTCTACC, reverse fixed region: CGTAAGTCCGTGTGCGAA). The starting library was designed with a A:C:G:T molar ratio of 3:3:2:2.4 to adjust for equimolar amounts of nucleotide incorporation and primers; forward primer: TCGCACATTCCGCTTCTACC, 5′-phosphorylated reverse primer: /5Phos/TTCGCACACACGGACTTACG. Complementary blocking regions were used during library preparation to block the primer regions during incubation steps (5′-complementary: GGTAGAAGCGGAATGTGCGA, 3′-complementary: TTCGCACACACGGACTTACG), similar to previous descriptions. Dynabeads M-270 Carboxylic Acid were purchased from Thermo Fisher Scientific (Waltham, MA), along with N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) required for magnetic bead activation. Binding buffer used throughout the selection was prepared with 20-mM tris-HCl (pH 7.4), 140-mM NaCl, 5-mM KCl, 1-mM MgCl₂, and 1-mM CaCl₂. Washing buffer was comprised of binding buffer supplemented with 0.005% Tween-20.

2.2 | Preparation of target-immobilized beads

1 × 10⁸ M-270 Carboxylic Acid Dynabeads were conjugated with PDGF-BB before each round of selection according to the manufacturer's instructions. The magnetic beads were washed with MES buffer (25-mM 2-(N-morpholino)-ethane sulfonic acid, pH 6.0), activated with EDC/NHS chemistry for 30 minutes, and incubated with PDGF-BB for 1.5 hours with rotation at room temperature. After incubation, the beads were washed with 50-mM tris-HCl (pH 7.4) buffer and incubated with the same buffer for 1 hour to ensure that all unreacted groups on the magnetic beads were quenched. The beads were finally washed with phosphate-buffered saline (PBS) buffer with 0.005% Tween-20 and suspended in binding buffer and stored at 4℃ until utilized. Beads were prepared fresh for each round of selection, and immediately prior to their use, beads were washed three times with wash buffer and resuspended in binding buffer.

2.3 | SELEX process

A single well of a hydrogel bonded, ultralow attachment 96-well plate (Corning, St. Louis, MO), was plumbed by drilling two holes into the lid, inserting dispensing needles (Jensen, North Andover, MA) and circulating fluid using a peristaltic pump (Fisher Scientific) (Figure 1). Manifold pump tubing (polyvinyl chloride, 0.51-mm ID, Fisher Scientific) was flushed with washing buffer containing 100 mg/mL of yeast tRNA (Thermo Fisher Scientific) and 0.1% bovine serum albumin (RPI, Mt. Prospect, IL) to passivate the lines before introducing the aptamer pools. Beads were trapped at the bottom of the plumbed well using a neodymium magnet. The selection was initiated with 1 nmole (~10¹⁴ sequences) of starting random DNA library. The starting library pool was mixed with equimolar amounts of complementary blockers, heated to 95℃ for 5 minutes, and slowly cooled down to 25℃ in a standard thermocycler at a rate of 0.5℃/min. Aptamer pools were circulated over the magnetically trapped beads at a rate of 20 mL/h. Wash buffer was then circulated at a rate of 50 mL/h to continuously remove unbound and weakly bound aptamers. After washing, aptamers bound to the beads were resuspended in 100 μL of ultrapure water and heated at 95℃ for 10 minutes to elute the bound nucleic acids. Round 1 only included a positive incubation step with PDGF-BB. Round 2 and beyond included both positive incubation and negative counterselection, where soluble PDGF-AA, -CC, and -DD were included in the wash buffer solution for the integrated counterselection step.
FIGURE 1  Systematic evolution of ligands by exponential enrichment (SELEX) workflow. (A) Conceptual overview of the integrated SELEX process. Iterative cycles of positive selection, combined washing, and negative selection, and emulsion polymerase chain reaction (PCR) and enzyme digestion are repeated to converge a starting nucleic acid library. When the pool is determined to have high affinity for the primary target, high throughput sequencing is used to identify individual aptamer sequences to test for affinity and specificity. (B) Schematic for the setup of the flow system. Manifold pump tubing (1) is inserted into dispensing needles (2). Dispensing needles are inserted through drilled out holes in the lid of a 96-well plate (3). The lid with dispensing needles is placed over the wells of a 96-well plate (4) where the micromagnetic beads (5) are immobilized by a magnet (6) underneath the plate. Fluid with either the aptamer pool or soluble off-targets are perfused unidirectionally through one dispensing needle and removed from the well through the other dispensing needle with the use of a peristaltic pump (not shown). (C) Schematic showing a positive selection round, where an aptamer pool is circulated over immobilized protein-conjugated magnetic beads. D, Schematic showing a negative selection step, where soluble off-target proteins are circulated over the aptamers bound to the primary target on the immobilized magnetic beads.

2.4  Emulsion PCR

Emulsion PCR protocols were modified for larger-scale preparations. Emulsion PCR oil was prepared in 20-mL glass scintillation vials with a mixture of 900 μL of Span-80, 80 μL of Tween-80 and 10 μL of Triton X-100 in mineral oil. The oil mixture was blended with a magnetic stir bar at 1500 rpm for 10 minutes in 4°C. The aqueous PCR mixture was prepared with 10x PCR buffer, 500-nM forward and reverse primers, 400-nM deoxyribonucleotide triphosphates (dNTPs), 5 U/μL of VAPRase enzyme (produced by Vanderbilt Antibody and Protein Resource [VAPR] core facility at Vanderbilt University), nuclease-free H2O, and 10 pM of template DNA from each round of SELEX. 1 mL of the aqueous PCR mixture was slowly added to 2 mL of the emulsion PCR oil over 2 minutes, while continuously spinning at 1500 rpm. The oil/aqueous PCR mixture was stirred for an additional 10 minutes at 4°C, continuously spinning at 1500 rpm. Once emulsions were formed, the 3 mL volume of emulsion mixture was pipetted into 30 separate PCR tubes (100 μL/tube) and run at optimized PCR settings on a standard thermocycler (2 minutes denaturation at 98°C, 15 cycles of 30 seconds of denaturation at 98°C, 10 seconds of annealing at 56°C, and 15 seconds of extension at 72°C, and final 5 minutes of annealing at 72°C). The PCR products were then pooled and broken with the use of 1-butanol. After adding butanol, the mixture was vortexed for 1 minute until the solution became clear, signifying that the emulsions were broken. The solution was centrifuged at 16 000g for 10 minutes at room temperature to pellet the DNA. The DNA pellet was then dried and resuspended in ultrapure water.

2.5  Regeneration of single-strand DNA

Emulsion PCR products were run on a 3% agarose gel to ensure correct band size and to check for any smearing from overamplification and high-molecular weight byproduct formation. Single-strand DNA was regenerated after PCR by incubating double-strand DNA with 5 U of λ-exonuclease (NEB, Ipswich, MA) at 37°C for 1 hour, followed by heat
inactivation at 75°C for 10 minutes in a standard PCR thermocycler.\textsuperscript{15} The single-strand product was purified through phenol/chloroform/isomayl alcohol extraction (Thermo Fisher Scientific) and ethanol precipitated. Single-strand DNA was resuspended in binding buffer before being used in the next round of selection.

### 2.6 Pool affinity characterization

After five rounds of selection, the eluted aptamer pool was prepared and characterized with a quantitative PCR (qPCR)-based bulk affinity assay.\textsuperscript{16} The round 5 pool was 2-fold serially diluted into eight concentrations spanning 200 to 0 nM. 2 × 10\textsuperscript{7} of PDGF-BB immobilized magnetic beads were added to each dilution and incubated at room temperature for 1 hour with gentle mixing using a spinning tube rotator. After the incubation, beads were washed three times with washing buffer and incubated at 95°C for 10 minutes in a standard heat block to elute bound aptamers. The eluted aptamer pools were mixed with iQ SYBR Green Supermix (Bio-Rad, Hercules, CA) and 250 nM of forward and reverse primers. The pools were amplified according to the manufacturer’s instructions: 30 seconds at 95°C and 40 cycles of 15 seconds of denaturation at 95°C and 30 seconds of annealing/extension at 60°C. Bound aptamer fractions were determined through comparison to a standard curve made from the starting DNA library. \( \Delta C_q \) values were nonlinearly fitted to the Langmuir equation to determine the bulk dissociation constant (\( K_D \)) of the round five pool. In addition, the round 5 eluted aptamer pool was characterized for its specificity to the primary target PDGF-BB against the surface of the magnetic beads. The round 5 pool and starting library were PCR-amplified with FAM-labeled forward primer and digested with \( \lambda \)-exonuclease. These ssDNA samples were incubated with either 2 × 10\textsuperscript{7} PDGF-BB immobilized magnetic beads or 2 × 10\textsuperscript{7} quenched beads for 1 hour. Beads were washed three times with washing buffer and bound aptamers were eluted from the beads at 95°C for 10 minutes in a standard heat block. The fluorescence intensity of the eluted sequences was measured with a standard plate reader.

### 2.7 High-throughput DNA sequencing

The round five-aptamer pool was prepared for sequencing on the Illumina HiSeq platform 2 × 150 bp configuration with ~350-M raw paired-end reads per lane (Genewiz, South Plainfield, NJ). To prepare the sample, the aptamer pool was amplified by emulsion PCR for 10 cycles to generate ~1-ug double-strand DNA. The sample was examined on a 2% agarose gel and extracted using the Qiaquick Gel Extraction Kit (Qiagen, Hilden, Germany). After sequencing, the data were analyzed using a custom script generated from Vanderbilt Technologies for Advanced Genomics Analysis and Research Design (Figure S1, Supporting Information). Raw sequences were cut around the forward primer and reverse primer fixed regions, which includes the 40-mer random region, and sorted based on relative abundance.

### 2.8 Affinity and specificity measurements

Single-stranded 40-mer DNA sequences lacking primer regions were ordered from IDT with a 5’-FAM fluorophore attachment. Affinity against each soluble protein was measured via microscale thermophoresis (MST) using the Monolith NT.115 device (Nanotemper Technologies, Munchen, Germany).\textsuperscript{19,20} Briefly, 16 2-fold serial dilutions of soluble protein were prepared from concentrations of 300 nM to 9.15 pM in binding buffer. 40-nM solutions of each 5’-FAM-labeled aptamer was prepared by diluting the aptamer in binding buffer, heating the mixture to 95°C for 5 minutes, snap-cooling on ice, and incubating at room temperature for 10 minutes. The folded aptamers were mixed with each dilution of protein and incubated in the dark for 1 hour at room temperature. Then, a standard Monolith NT.115 capillary was dipped into each serially diluted solution and fluorescent dose-response was measured at 20% MST excitation power. Equilibrium binding constants were fitted using the MO. Affinity Analysis software (Figure S2). Specificity is reported as affinity for the primary target vs each individual off-target.

### 2.9 Ethics statement

All work involving recombinant DNA was approved by the Vanderbilt University Institutional Biosafety Committee.
3 | RESULTS AND DISCUSSION

3.1 | Choice of target and outline of SELEX procedure

To facilitate our studies, we chose the PDGF family as a model system, with PDGF-BB used as the primary target and PDGF-AA, -CC, and -DD, as the off-targets. Aptamers have previously been reported against PDGF-BB with binding affinities in the low nanomolar range, suggesting its utility as a model target. In particular, microfluidic SELEX coupled with HTS has been used to identify aptamers that bind to PDGF-BB with high affinity and moderate specificity relative to PDGF-AA. Thus, using the PDGF family, our SELEX approach can be quantitatively benchmarked against other methods.

As previously stated, our selection scheme was designed to integrate certain concepts from other SELEX procedures into a workflow that is manageable without highly specialized techniques or equipment. First, we incorporated fluid flow for each step of the selection using magnetic immobilization of targets and an inexpensive peristaltic pump, building off concepts established in microfluidic devices (Figure 1). Flow also allows for the recirculation of the library and subsequently enriched nucleic acid pools over the immobilized target; in this selection scheme, the total volume is circulated through the system roughly five times in 1 hour, thus yielding multiple opportunities for the prospective aptamers to interact with the target. In addition, as previously described, fluid flow increases the stringency of the washing steps to remove weakly bound aptamers from the primary target. To validate the initial flow rate and circulation time used for SELEX, we employed a previously reported aptamer against PDGF-BB and analyzed real-time binding via fluorescence. In these experiments, the PDGF-BB aptamer was labeled with a fluorophore, diluted in binding buffer to a concentration of 2 nM, and circulated over three wells of a 96-well plate at 20 mL/h. One well contained immobilized PDGF-BB, the second well contained immobilized mouse IgG, and the third well was fully passivated without any added protein. The aptamer was circulated for 60 minutes and fluorescence was measured every 10 minutes by disconnecting the plate from the pump and assaying on a plate reader (each well was briefly washed with washing buffer before every measurement) (Figure 2). After the first reading, fluorescence was noticeably increased in the well where PDGF-BB was immobilized but not in the control wells. Within 20 minutes, the fluorescence in the PDGF-BB well reached a plateau, potentially indicating that all of the PDGF-BB target proteins in the well were saturated with bound aptamer, whereas no significant changes in fluorescence were observed in the control wells throughout the remainder of the experiment. This result validated the ability of high-affinity aptamers to bind a target under active flow and demonstrated that unbound aptamers are not retained against nonspecific targets.

Next, we incorporated emulsion PCR for all amplification steps of the workflow to reduce byproduct formation and high-molecular weight artifacts. Several research groups have incorporated the use of emulsion PCR into their selection workflow to improve the yield of product amplification and reduce the formation of larger molecular weight byproducts that are evident after high cycle amplification of random nucleic acid libraries. Emulsion PCR has also been shown to reduce the amount of amplification artifacts and byproducts through rounds of amplification. This selection workflow uses emulsion PCR in every round of selection as described in the methods section. Byproduct formation from amplification of random nucleic acid libraries can be observed on an agarose gel after only 10 cycles, whereas our adjusted emulsion PCR protocol does not show evidence of byproduct formation, even up to 35 cycles (Figure 3A,B). Following emulsion PCR amplification, the dsDNA aptamers were restored to ssDNA with a λ-exonuclease enzymatic digestion, as previously described (Figure 3C).
Finally, we utilized a consolidated counterselection strategy, where off-targets are introduced into the system during washing steps and, therefore, exposed to prospective aptamers that are still bound to the primary target. In this manner, the aptamers bound to the immobilized target are exposed to all off-targets simultaneously during the negative selection, providing a competitive binding atmosphere to enrich for specificity. This approach eliminates the need for separate rounds of counterselection, thereby benefiting SELEX workflows by shortening the overall procedure.

3.2 | SELEX workflow

Five rounds of selection were completed with the PDGF family of proteins as described in the methods section. The first round of selection performed only involved a positive selection with the primary target PDGF-BB. In rounds 2 to 5, a negative selection step was performed immediately after positive selection by including PDGF-AA, -CC, and -DD during the washing stage. Through the five rounds, the amount of primary target and the time of positive incubation were decreased, while the amount of off-targets, the time of washing, and the time of negative selection were increased (Table S1). The aim was to increase the ratio of the primary target to off-targets to increase the selection pressure through each round of selection, eventually increasing to a ratio of 1:100. After the fifth round of selection, we labeled the enriched pool with a FAM fluorophore to show preferential binding against PDGF-BB-coated beads but not quenched beads (Figure 4A). The pool affinity toward PDGF-BB was then assayed with a qPCR assay. The bulk pool affinity was measured to be $24 \pm 17$ nM, compared to the starting library which had insignificant binding to PDGF-BB (Figure 4B).

3.3 | Identification and characterization of prospective aptamers

We utilized HTS and bioinformatics analysis to identify the most abundant aptamers in the round 5 pool; as described earlier, this approach is becoming increasingly common for identifying high-affinity aptamers without pool convergence and laborious molecular cloning.\(^7\) A total of 8,171,212 prospective aptamer sequences were identified, and analysis revealed that the pool was not highly converged after five rounds of selection, with only 11.91% of the pool represented as duplicate sequences. The most abundant sequence represented 2.52% of the total population of the round 5 pool, with the top
FIGURE 4  Bulk affinity of aptamer pool after five rounds of selection. (A) Binding experiment measuring mean fluorescence intensity of the round 5 pool to platelet-derived growth factor (PDGF)-BB-coated beads and quenched beads, compared to the starting library. Data are presented as mean ± SD from technical triplicates. (B) quantitative polymerase chain reaction (qPCR) measurements of dissociation constants in the round five-aptamer pool and starting nucleic acid library. The plots represent a single qPCR run, and results were verified across biological triplicates. The average $K_D$ of the round five pools is 24 ± 17 nM.

FIGURE 5  Affinity of individual aptamers for platelet-derived growth factor (PDGF)-BB. The most abundant sequences from high throughput sequencing (HTS) were measured for affinity to PDGF-BB via microscale thermophoresis (MST) binding measurements (A)-(F). The plots represent binding isotherms from triplicate MST experiments, with final affinities reported as mean ± SD.

10 sequences representing less than 7% of the total population (Table S2). In addition, there was little structural homogeneity between the top sequences analyzed in this study (Figure S3). The most abundant sequences from the pool were tested for their affinity to PDGF-BB using MST, and the sequences with the highest affinity for PDGF-BB were also tested for their specificity against PDGF-AA, -CC, and -DD. The 10 assayed aptamers possessed affinities for PDGF-BB ranging from 49 to 155 nM, compared to the affinity of 16 nM of the previously reported aptamer that was used to validate initial flow rates (Figure 5). The aptamers were then tested for their specificity against the other homodimers used in the counterselection step of the workflow (Table S3). Overall, the aptamers exhibit moderate specificity against the off-targets. For example, one aptamer, PDGF-2, with an affinity of 49 nM to primary target PDGF-BB, exhibited 2.3-, 11.8-, and 10.7-fold specificities against off-targets PDGF-AA, -CC, and -DD, respectively (Figure 6B). Another aptamer, PDGF-6, with an affinity of 50 nM to primary target PDGF-BB, exhibited 6.0- and 5.6-fold specificities against off-targets PDGF-AA and -CC, with no change in specificity against PDGF-DD (Figure 6C). Binding specificities of the other aptamer sequences...
FIGURE 6  Specificity measurements. Select aptamers were measured for affinity to platelet-derived growth factor (PDGF)-AA, PDGF-CC, and PDGF-DD via microscale thermophoresis (MST) binding measurements. The plots represent binding isotherms from single MST experiments, and the final affinities are mean ± SD from biological triplicates. (A) A2 aptamer from a previous study. (B) Aptamer PDGF-2 from this study. (C) Aptamer PDGF-6 from this study.

are reported in Figure S4. Meanwhile, the previously reported aptamer, A2, exhibited 5.0-, 24.0-, and 12.0-fold specificities against PDGF-AA, -CC, and -DD, respectively (Figure 6A), which are reasonably similar to aptamer PDGF-2 in this study.

We do note that specificity for the selected aptamers in this study seems to be stochastic. On the other hand, we used three off-targets during counterselection, and in most cases, specificity was only observed against one or two of the off-targets. Interestingly, the selection scheme for A2 only included PDGF-AA during counterselection, yet this aptamer exhibits specificity over PDGF-CC and PDGF-DD similar to the aptamers from our study. Thus, while our results do indicate that the competitive counterselection approach is effective, more steps need to be taken to further drive specificity. We expect to explore modulation of the counterselection parameters and other variables that could impact selection fidelity (such as flow rates) in future studies.

4  | CONCLUSIONS

In this work, we describe a selection approach that builds off previous SELEX advancements, as well as incorporates a consolidated counterselection strategy, thereby generating high-affinity and moderate specificity aptamers. We recently reported the design of a custom-built multichannel peristaltic pump that could be used for this purpose, continuing on the theme of bringing novel ideas and low-cost resources to the aptamer community. Overall, once improvements are made to the selection workflow to better impart specificity, we envision that such an array-based system could provide diverse sets of aptamers for researchers aiming to develop therapeutic and diagnostic tools.
ACKNOWLEDGMENTS
The authors would like to thank Dr. Y. Guo for assisting with HTS data analysis, Mr. M. Rahim for helping with analyzing HTS data, and Dr. O. Koues for advisement on HTS workflows. Technical support was provided by the Vanderbilt VANTAGE core facility, which is supported in part by CTSA Grant SUL1 RR024975-03, the Vanderbilt Ingram Cancer Center (P30 CA68485), the Vanderbilt Vision Center (P30 EY08126), and NIH/NCRR (G20 RR030956). Funding for this work was provided by an Engineering Immunity Pilot Grant from Vanderbilt University and an American Cancer Society Institutional Research Grant (IRG-58-009-56). DAB was supported by the Vanderbilt University Medical Scientist Training Program (T32 GM007347).

CONFLICT OF INTEREST
The authors have no conflict of interest relevant to this article.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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**How to cite this article:** Rosch JC, Balikov DA, Gong F, Lippmann ES. A systematic evolution of ligands by exponential enrichment workflow with consolidated counterselection to efficiently isolate high-affinity aptamers. *Engineering Reports*. 2020;2:e12089. [https://doi.org/10.1002/eng2.12089](https://doi.org/10.1002/eng2.12089)