

*Research Article*

# Aptamers in Education: Undergraduates Make Aptamers and Acquire 21<sup>st</sup> Century Skills Along the Way

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**Abstract:** Aptamers have a well-earned place in therapeutic, diagnostic, and sensor applications, and we now show that they provide an excellent foundation for education, as well. Within the context of the Freshman Research Initiative (FRI) at The University of Texas at Austin, students have used aptamer selection and development technologies in a teaching laboratory to build technical and 21st century skills appropriate for research scientists. One of the unique aspects of this course-based undergraduate research experience is that students develop their own projects, and take ownership of their own science in what would otherwise be a traditional teaching lab setting. Of the many successes, this work includes the isolation and characterization of novel calf intestinal alkaline phosphatase (anti-CIAP) RNA aptamers by an undergraduate researcher. Further, preliminary survey data suggest that students who participate in the aptamer research experience express significant gains in their self-efficacy to conduct research, and their perceived ability to communicate scientific results, as well as organize and interpret data. This work will describe the use of aptamers in an educational setting, highlight the positive student outcomes of the aptamer research experience, and more particularly present the research findings relative to the anti-CIAP aptamer.

**Keywords:** aptamer; calf-intestinal alkaline phosphatase; course-based undergraduate research experience; in vitro selection; Systematic Evolution of Ligands by Exponential Enrichment; SELEX; CURE; Freshman Research Initiative; 21<sup>st</sup> Century Skills.

## 1. Introduction

With a 29-year history, aptamers have a well-established presence in diagnostic, therapeutics, and sensor technologies [1-3]. Furthermore with time, the range of aptamer applications has broaden with some applications stretching the field in interesting ways, including their use as molecular recognition elements (e.g. ELISA/ELONA, [4]), imaging elements (e.g. within live cells, [5]), DNA origami and nanorobots [6], and now aptamers have taken a foothold in education. As students increasingly choose to explore engineering approaches to biology, often lumped under the mantle of “synthetic biology,” an educational introduction to aptamers provides one of the best means for fomenting their interest and providing them with the skills needed for professional success.

Aptamer technological and scientific advancements have been adopted and adapted to long-term course-based undergraduate research experiences (CURE). Meeting the urgent call for authentic research experiences in education [7-9], CURES have emerged throughout STEM fields. For example, with the emergence of smart phone technologies and sensor peripherals, a CURE devoted to DIY Diagnostics was developed (University of Texas, UT-Austin). Similarly, with the advent of data analytics, the University of Maryland’s Sustainability Analytics CURE was established. Likewise,

CUREs using CRISPR (UT-Austin), nanochemistry (UT-Austin), biomaterial design (Iowa State University), as well as other technologies and advancements have emerged across the country.

Critical to these authentic research experiences for undergraduates is the institutional support of undergraduate research programs, such as through the Freshman Research Initiative (FRI, UT-Austin, UT Rio Grande Valley, and Iowa State University), the First-year Innovation & Research Experience (FIRE, University of Maryland), Program to Educate and Retain Students in STEMS Tracks (PERSIST, UT El Paso), and the Achieving Success through Undergraduate Research and Engagement (ASSURE, UT Arlington).

UT-Austin launched the FRI in 2006 with three CUREs. An aptamer selecting CURE, named the Aptamer Stream, was among these original teaching research labs. As of 2019, in its thirteenth year, the FRI includes 29 different CUREs offering authentic research experiences across a variety of disciplines and research areas, including robots, nanomaterials, computer security, organic chemistry, molecular biosciences, and more. Approximately 900 freshman students at The University of Texas participate in this program annually, with 50% of the students from underrepresented minorities [10].

Central to CURES and the supporting undergraduate research programs are the undergraduate students and the desire to positively impact and retain them over time. Previous outcomes of such initiatives have shown that participation in CURES leads to a 17% increased college graduate rate in 6 years; a 23% increased likelihood of earning a science, engineering, or mathematics degree (UT-Austin FRI, [11]); increased student interest in science, college retention, and science course grade (SEA Phage Hunters Program, [12]); increased independence and knowledge gains [13]; and, increased confidence and understanding of the research process [14-15], and, in the case of the UT-Austin FRI students, an estimated 16% more in lifetime earnings [16].

Through the UT-Austin FRI (briefly described in [17]), the Aptamer Stream students participate in a year long, two-semester (spring to fall with an optional summer session) research experience utilizing aptamers under the guidance of a non-tenure track faculty member (co-author Stovall, G.M.), a tenure-track principal investigator (co-author Ellington, A.D.), and near-peer mentors. In the first spring semester, approximately 35 freshman students perform *in vitro* aptamer selections using parallel methodology, while receiving lower-division chemistry lab course credit, students begin their technical training in the beginning of the first semester, performing an aptamer selection (such as against egg-white lysozyme). In the latter half of the first semester, students begin a new aptamer selection against one of the provided targets. Students have the option to continue their research over the summer, where they may progress on their aptamer selections and assay the selected pools for sequence enrichment (i.e. sequencing) and/or binding. The traditional FRI sequence concludes in the fall semester of students' sophomore year. During this fall semester, students receive lower-division biology course credit, continue their aptamer selection, assay their selected pool, characterize aptamers, and/or develop an aptamer application.

Integrating aptamer research into a teaching lab, the Aptamer Stream research experience is at its core authentic, with long-term research goals to identify aptamers and develop aptamer applications. Signifying a new endeavor in education, this work describes the use of aptamers in an undergraduate research setting, identifies and characterizes novel anti-calf intestinal alkaline phosphatase (CIAP) aptamers identified in the Aptamer Stream, and highlights some of the educational student benefits from such a research experience.

## 2. Materials and Methods

### *Integration of aptamer research into a teaching lab*

The integration of aptamer research into an educational setting is at the forefront of all aspects of the Aptamer Stream. At the start of the course, the learning objectives and skills articulate the dual-purpose of the course, which includes aptamer research and 21st century learning skill development. For example, in the fall of 2018, students in the Aptamer Stream were assessed on the development of the following skills through the described learning objectives [18]:

Students will develop:

1. A fundamental awareness and early experience in scientific research, specifically in the field of aptamer development (oligonucleotide affinity reagent development). This involves an introduction to the terminology, technical concepts, and principles of the research. The learning objectives include:
  - a. Identify a creative, focused, and manageable research question or topic.
  - b. Design a methodology for answering a research question, perusing the project, or small-scale “troubleshooting” tests.
  - c. Demonstrate the understanding of the research implications and its translation to practical applications.
2. Teamwork
  - a. Brainstorm troubleshooting and/or problem-solving ideas with other students and/or mentors.
  - b. Make changes to work based on critical analysis of work and on peer review feedback.
3. Communication
  - a. Develop and practice scientific writing skills.
  - b. Develop science communication skills, as well as further develop argumentation skills, including the connection between the problem and the solution.
4. Data Analysis Experience
  - a. Construct a meaningful figure using research data, which includes appropriate controls and statistics, if appropriate.
  - b. Collect, interpret, evaluate, provide context, and rational conclusions for research data.
5. Resilience
  - a. Develop and implement mechanisms to overcome, bypass, and/or wade through setbacks.
  - b. Initiate projects or activities with set deadlines and sometimes incomplete information.

Through the Aptamer Stream two-semester experience, students (approx. 35 students/semester) are trained on the technical aspects of *in vitro* aptamer selections in the first semester. Midway through the first semester, students select one target from a list of options to begin their independent research project to identify aptamers against that target. The *in vitro* aptamer selections continues through the summer (optional) and the following fall semester to conclude the formal FRI sequence of courses. Many students, however, continue their research experience as independent researchers and sometimes in addition to serving as near-peer mentors for the future student cohorts.

In the Aptamer Stream, the technical training involves the iterative *in vitro* RNA aptamer selection process using bead-based (described below) and filter-based methodologies [19]. In general, after four to six rounds of RNA aptamer selection, the selected RNA pools are cloned and Sanger sequenced to examine sequence or motif enrichment. <sup>32</sup>P-radiation binding assays are performed (described in [20]). (For specific information regarding the selection of the anti-CIAP aptamer, please see “Materials and Methods: Anti-CIAP” sections below.)

To meet the educational objectives of the lab, the Aptamer Stream students meets weekly in a lecture-style class, weekly in a less formal small group/discussion group meetings, as well as 6 or 8 hrs/wk in lab in the spring and fall semesters respectively. Weekly lectures in the spring include content-specific materials related to the *in vitro* aptamer selection methodology (e.g. PCR, gel electrophoresis, oligonucleotide purification and quantification, transcription, etc.), as well as aptamer applications and technologies. Weekly lectures in the fall primarily include student presentations, with the occasional content lecture.

The research described here was performed in a 1,500 sq ft (approx.) FRI lab space that houses two FRI CURES (i.e. Aptamer Stream and Virtual Drug Screening Stream), as well as the equipment necessary to conduct molecular biology research. The space accommodates 25 to 30 (approx.) people at one time and includes room for instrumentation, bench space for 32 researchers, one fume hood, and one biosafety cabinets adequate for BSL2 work. The laboratory is equipped with one orbital shaker for bacterial growth, a Beckman Allegra X15-R centrifuge for oligonucleotide pool preparation and precipitations, as well as bacterial and protein preparation, two micro-spectrophotometers

(nanodrop spectrophotometer) for sample quantification, a BioTek Synergy HT plate reader (96-well), a Savant ISS110 SpeedVac Concentrator, 10 thermocyclers, 4 bench-top centrifuges, an incubator, 11 microcentrifuges, 2 analytical balances, multiple pH meters, multiple heat plates, 7 benchtop vortexers, 4 benchtop water baths, 2 Max Q 7000 water baths, multiple gel electrophoresis apparatuses and gel dryers, an ultrafreezer, a Barnstead nanopure water dispenser, and all the refrigerators, and materials necessary for molecular biology research.

#### *Anti-CIAP Aptamer Research: in vitro Aptamer Selection (RNA aptamers)*

*In vitro* aptamer selection methodology or Systematic Evolution of Ligands by Exponential Enrichment (SELEX) was utilized to enrich for anti-CIAP aptamers. In general, biotinylated-CIAP (69 kDa, Pierce Thermo Fisher, catalog no. 29339) and pool were employed in a toggle selection, using streptavidin bead-based selection and a filter-based selection methodology [9] to enrich anti-CIAP aptamers. The N50 RNA pool (97 nt) was used in the *in vitro* anti-CIAP aptamer selection,

5'-GGGUUUACCUAGGUGUAGAUGCU-N50-AAGUGACGUCUGAACUGCUUCGAA-3'

Refer to Table S1: Anti-CIAP Aptamer Sequences for a text file of all oligonucleotides used in this research.

For example, in round 1, 50  $\mu$ L of streptavidin beads (Fisher Dynabeads M-270 Streptavidin, catalog no. 65305) were washed with water, then incubated for 30 min at room temperature with 400 pmol of biotin-CIAP, and then washed with PBS. After heat denaturing and cooling to room temperature, the 400 pmol of natively folded N50 RNA pool was incubated with the CIAP-beads (round 1) at room temperature for 25 minutes (rounds 1-6) or 15 minutes (rounds 7-9). After the incubation, several washes with selection buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 5 mM MgCl<sub>2</sub>) were performed to remove the weakly bound RNA species. For round 1, the total wash volume was 2.4 mL. In an effort to minimize the non-specific RNA binders and enrich CIAP-specific RNA binders, the selection stringency was increased each round by increasing wash volumes, performing negative selections, as well as increasing the selected RNA to CIAP ratio (i.e. decreasing CIAP from 400 pmol to 50 pmol). After the last wash, a negative selection was performed by removing the CIAP-beads and RNA into a new tube, thus removing RNA plastic tube-binders. RNA bound to the CIAP was eluted using 80°C nanopure water. Negative bead selections were performed every round (except 1 and 5) prior to incubation with the CIAP-beads by exposing the RNA pool to naked beads (i.e. without CIAP), thus removing bead-binding RNA. The collected washes and eluted RNA were ethanol precipitated. See Appendix A, Table 1 for specific selection conditions.

To amplify and analyze the RNA pool collected in washes and eluted for each round, the DNA analog was prepared and amplified. The selected N50 RNA pool was subjected to a reverse transcription reaction with the reverse primer:

"24.N50.R" primer 5'-TTCGAAGCAGTTCAGACGTCACCTT-3'

The subsequent cDNA was then amplified in various PCR reactions, which included a forward primer that reappended the T7-promotor (T7-promoter noted in bold):

"42.N50.F" primer 5'-GATAATACGACTCACTATAGGGTTTACCTAGGTGTAGATGCT-3'

In general, a cycle course PCR [10] was used to determine the effective ratio of non-binding RNA to CIAP-bound RNA (thus evaluating the stringency of the selection), as well as determine the necessary PCR cycles for sufficient amplification. A subsequent large-scale PCR reaction was performed and then concentrated via ethanol precipitation.

Recapitulating the selected RNA, a transcription reaction was performed, incubated for 2 hours at 42°C or overnight at 37°C. RNA was PAGE purified using UV shadowing. Finally, the selected RNA was eluted from the PAGE gel chunks overnight in 10 mM Tris-HCl, pH 8.0 and 1 mM EDTA (TE) buffer or by the crush soak method, subsequently ethanol precipitated, resuspended in selection buffer, and spectrometrically quantitated in preparation for the next iterative round of aptamer selection.

#### *Anti-CIAP Aptamer Research: Sanger Sequencing and Aptamer Binding Assay of Selected Pool*

Using a TA Cloning kit (Thermo Fisher Scientific Invitrogen, catalog no. K2020-20), PCR product from selected rounds was cloned into the pCR 2.1 vector and Sanger sequenced using M13 primers. Sequence enrichment was determined by visually inspecting sequence data for recurring clones and motifs.

To ascertain the RNA to CIAP binding enrichment over multiple rounds of aptamer selection, a  $^{32}\text{P}$  radiation binding assay was performed similarly to that described previously [20]. Recurring RNA clones in the selected pool (i.e. potential aptamers), as well as the minimized variant, were tested as in such a binding assay.

### *Educational Assessment Methods*

To track and monitor the impact of this authentic research experience on students, both formative and summative assessments are conducted each semester and annually at both the research stream level and at the FRI programmatic level. The formative assessments provide instructors with student feedback to gauge what is working well and what needs improvement. The data gleaned from feedback forms conducted each semester inform any mid-course corrections and provide instructors with actionable information to enhance the experience for students. The summative assessments examine how the experience in the aptamer research stream impacted students' psychosocial attitudes towards science and science research. Pre/post surveys are administered each semester to track student growth over time. Specifically, the survey draws on previous, empirically validated measures designed to assess: Science Self-Efficacy [21], Identity and Belonging in Science [21-22], Grit/Resilience [23-24], and Intention to Persist [25] and Preparedness [23].

In addition to assessing psychosocial attitudes, the pre/post survey also captures students' self-reported gains in research skills from pre to post. Specifically, the following 21<sup>st</sup> Century Learning Skills [26] were assessed:

- *Effective Communication*: the ability to produce written and oral reports, and make persuasive, evidence-based arguments using appropriate scientific sources and effective figures and graphics.
- *Information Literacy*: the ability to locate appropriate information, evaluate sources critically, read and interpret primary scientific literature, and synthesize information.
- *Computational/Technological Literacy*: the ability to organize and interpret data and apply computational skills to solve problems.
- *Self-directed Learning*: the ability to execute an independent and original project culminating in a product, such as a written document, oral presentation, or physical object; the ability to innovate, create, or conduct original research projects.
- *Teamwork*: the ability to resolve conflicts, plan, and coordinate group efforts.

In Fall 2017, a pre/post in-class student survey was deployed to approximately 24 students participating in the Aptamer Stream. The survey included items reflecting the constructs described above (e.g., Science Self-efficacy: "I have a lot of confidence when it comes to doing STEM research."). Students were asked to rate each item on a 5-point Likert scale (1, Strongly Disagree to 5, Strongly Agree). The data gleaned from the surveys were used to both track students' growth over time and to assess the extent to which the learning objectives of the course were met (e.g., Skill: Teamwork).

## **3. Results**

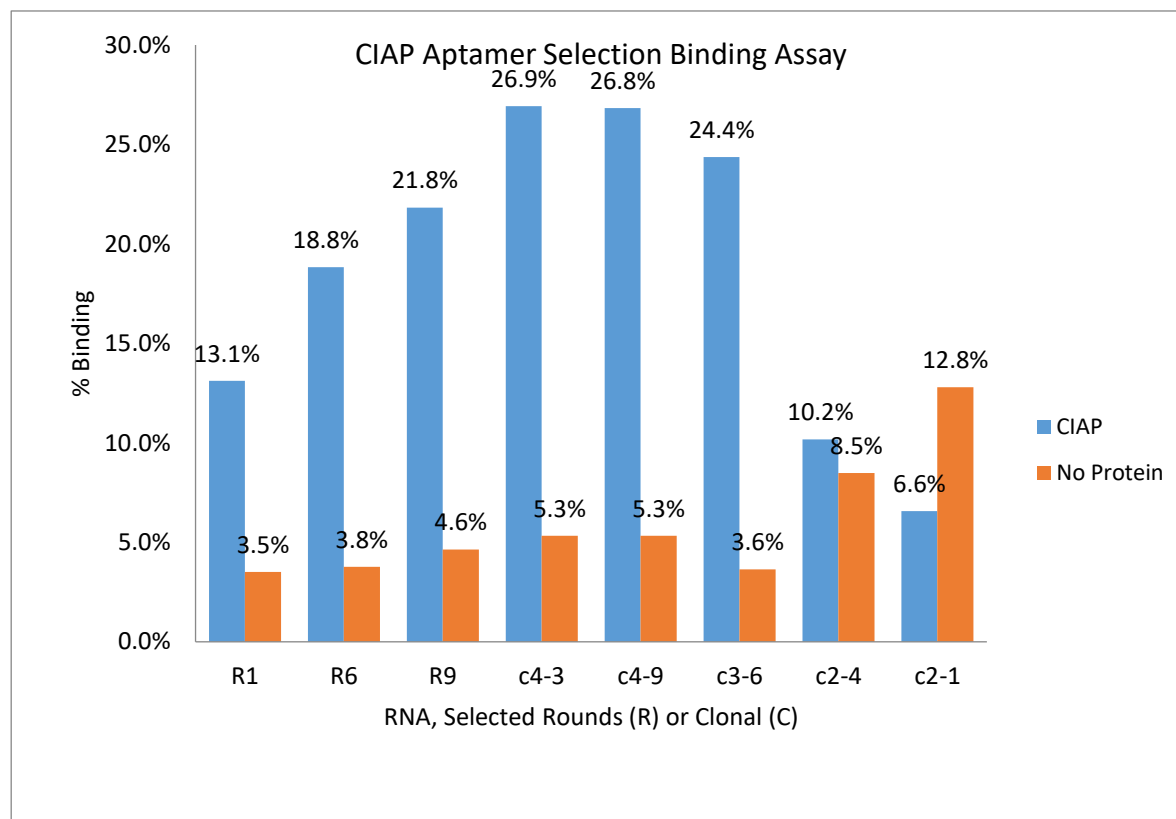
### *3.1 Anti-Calf Intestinal Alkaline Phosphatase (CIAP) Aptamer Research Results*

#### **3.1.1 Anti-CIAP Aptamer Selection and Sequence Enrichment Results**

Nine rounds of *in vitro* aptamer selection using an N50 RNA pool were performed to identify RNA aptamers against calf-intestinal alkaline phosphatase (CIAP). Early radiation binding assays revealed CIAP-binding enrichment in selected RNA from rounds 4 and 6 over round 1 (see Appendix B, Figure 1). Subsequently, three additional rounds, 9 rounds in all, of *in vitro* aptamer selections were performed with a decreased RNA:CIAP incubation time and increased stringency (see Appendix A,



Table 1), further challenging the CIAP-binders with increased washes and increased RNA pool: CIAP ratio.



**Figure 1.** Anti-CIAP aptamer selection radioactive binding assay results (representative experiment without replicates). RNA clones c4-3, c4-9, and c3-6 contained the 14-mer motif and had the greatest binding affinity to CIAP. RNA clones c2-4 and c2-1 did not contain the 14-mer motif and, as observed in the graph, had the lowest binding to CIAP and the highest binding to beads (“no protein”).

The selected RNA pool from round 9 was cloned, Sanger sequenced, and the sequencing data was visually inspected for sequence enrichment through the presence of recurring clones and sequence motifs. Of the 36 clones examined, two of the RNA clones were present twice and with a 13 nt motif (GAACUCAACAUAA) present in 19 of the examined sequences. Of these 19 sequences, 15 contained a G at the end of the motif and 4 contained an A at the end, thus the motif was extended to a 14 nt motif with the representation of a punctuating purine (GAACUCAACAUAAAR). When folded, 18 of the 19 clones containing the recurring motif present it in the loop structure (see Table 1).

### 3.1.2 Anti-CIAP Aptamer Minimized Variant Design, Assay Results, and Dissociation Constants

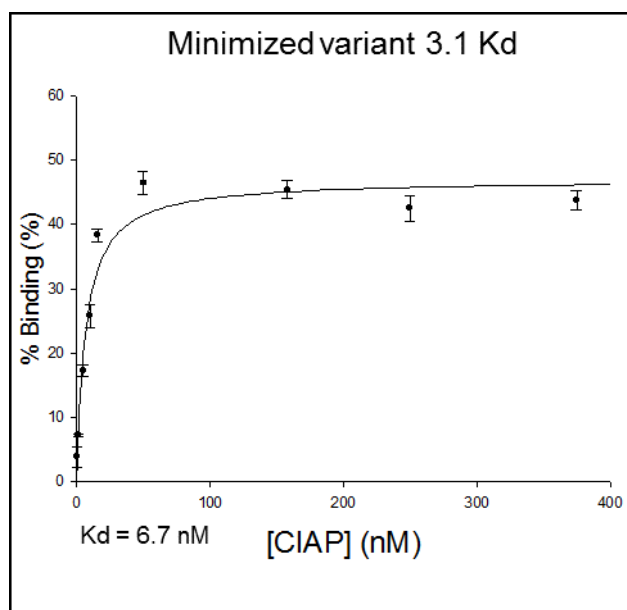
Testing selected RNA pools (i.e. rounds 1, 6, and 9) and recurring clones in a  $^{32}\text{P}$  radioactive binding assay revealed increased CIAP-binding, relative to the “no protein” beads, over multiple rounds (see Figure 1). RNA clones c4-3, c4-9, and c3-6, which contained the 14-mer VDH motif, demonstrated significant CIAP-binding over the “no protein” beads, binding more than the round 9 selected RNA pool. However, clones without the VDH motif (e.g. c2-4 and c2-1, see Table 2) bound to CIAP similarly as the RNA clones bound to the “no protein” beads (see Figure 1) and were thus eliminated as potential CIAP aptamers (Table 2).

**Table 2.** RNA clones without the VDH 14-mer motif. Binding assay data later eliminated these clones as potential anti-CIAP aptamers as they non-discriminately or preferentially bound naked beads (see Figure 1).

	Sequence
c2-4	GGGUUUACCUAGGUGUAGAUGCUUCUUCACUCCUUAUGAACACGUAGCGCUCAAUCAU CUCUAAUUAUUUCUCAAGUGACGUCUGAACUGCUUCGAA
c2-1	GGGUUUACCUAGGUGUAGAUGCUGAUAGAUUCCCCUGACUAGGAGCUCAUUAGAUU UUUAUUGUGUGGGGCAAGUGACGUCUGAACUGCUUCGAA

In an effort to rationally design a minimized binding variant, clone 4-3 was selected from the three clones tested (i.e. c4-3, c4-9, and c3-6, see Table 1) because initial binding assays indicated the greatest percentage of this clone bound CIAP (Figure 1). Using the mFold structure [27], the minimized variant was designed to preserve the VDH motif and the neighboring structural features (i.e. the primary loop and stem), while minimizing the distal structural elements. Given this design approach, the minimized variant 3.1 contained approximately more than half of the 5' pool static region (aka forward primer region), most of the random region, and none of the 3' static region (aka reverse primer region). Furthermore, to enhance transcription using a T7-promoter, a GGG was added at the beginning of the construct with a complementary CCC at the 3' end, thus contributing to the stem structure (see Table 1), and forming the minimized variant 3.1.

Using  $^{32}\text{P}$ -RNA binding assay, the dissociation constant of the minimized variant 3.1, as well as clones c4-3, c4-9, and c3-6, was determined to be 6.7 nM (see Figure 2), 5 nM, 9.4 nM, and 10.8 nM, respectively (Appendix C).



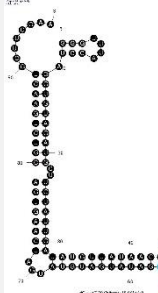
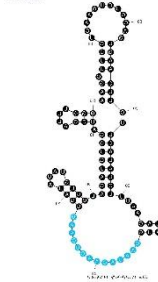
**Figure 2.** Dissociation constant (Kd) of minimized anti-CIAP RNA aptamer variant 3.1 and CIAP.

### 3.1.3 Anti-CIAP Aptamer Specificity and Activity

Assaying for binding specificity, the aptamer binding affinity against many CIAP-related proteins (such as human intestinal AP, human tissue-nonspecific AP, and bacterial AP) was examined and no significant binding was observed in radioactive binding assays up to 250 nM protein concentrations (see Appendix D).

Early kinetic assays examining aptamer inhibitory effects on the reaction of CIAP and its substrate p-nitrophenyl phosphate indicate little if any loss in alkaline phosphatase activity (data not shown). A follow-up kinetic study is suggested to conclude these findings.

**Table 1.** Recurring clones and motifs identified in the anti-CIAP *in vitro* aptamer selection (round 9). The bold sequences identify the former “random region” of the original N50 RNA pool and the non-bold/regular-type sequences identify the static regions designed for primer annealing. Note the presence of an R (i.e. A or G) in the VDH 2.14 motif and the presence of a W (i.e. A or T) in aptamer clone 4-9. Refer to Table S1: Anti-CIAP Aptamer Sequences for a text file version of this table.

MOTIF OR CLONE	%	Kd	FOLD AND NOTES
Motif VDH 2.14, 14 nt: GAACUCAACAUAR	53%	Not tested	 <p>VDH 2.14 motif appears in the loop structure (as seen in c2-2) approx. 18 out of 19 clones. Note the presence of an R (A or G).</p>
Aptamer Clone 4-3, 97 nt: 5' GGGUUUACCUAGGUGUAGAUGC <b>GUAAUUAAGCGAACUCAACAUAAAGGUA</b> <b>UAAUUACAAUUUCUAUACUUCUUC</b> AAGUGACGUCUGAACUGCUUCGAA3'	6%	5 nM	 <p>VDH 2.14 motif</p>

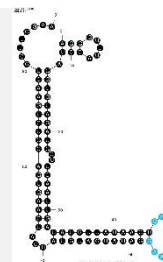


appears in the loop structure (as seen in c4-3) approx. 2 out of 2 times.

Aptamer Clone 4-9, 97 nt:

5' GGGUUUACCUAGGUGUAGAUGCU**UCWAUUGAUUAUGUUUAACUGAACUC**  
**AACAUAAGGAUAUGAUGUAUGAUC**AAGUGACGUCUGAACUGCUUCGAA3'

6% 9.4 n M



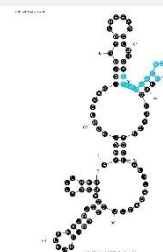
VDH 2.14 motif appears in the loop structure (as seen in c4-9) approx. 2 out of 2 times.

Note the presence of a W (A or T).

Aptamer Clone 3-6, 97 nt:

5' GGGUUUACCUAGGUGUAGAUGCU**CUGCCCUUCAGAUUUUAUCGAUGACCG**  
**UUGAACUCAACAUAAGACCUCCA**AAGUGACGUCUGAACUGCUUCGAA3'

3% 10.8 n M



VDH 2.14 motif is present in the loop and stem structure.

Minimized Aptamer Variant 3.1, 55 nt:

5' GGGUAGAUGCUGUAUAUAGCGAACUCAACAUAAAGGUAAAUUACAAUUU  
CUACCC3'

N/A 6.7  
n  
M



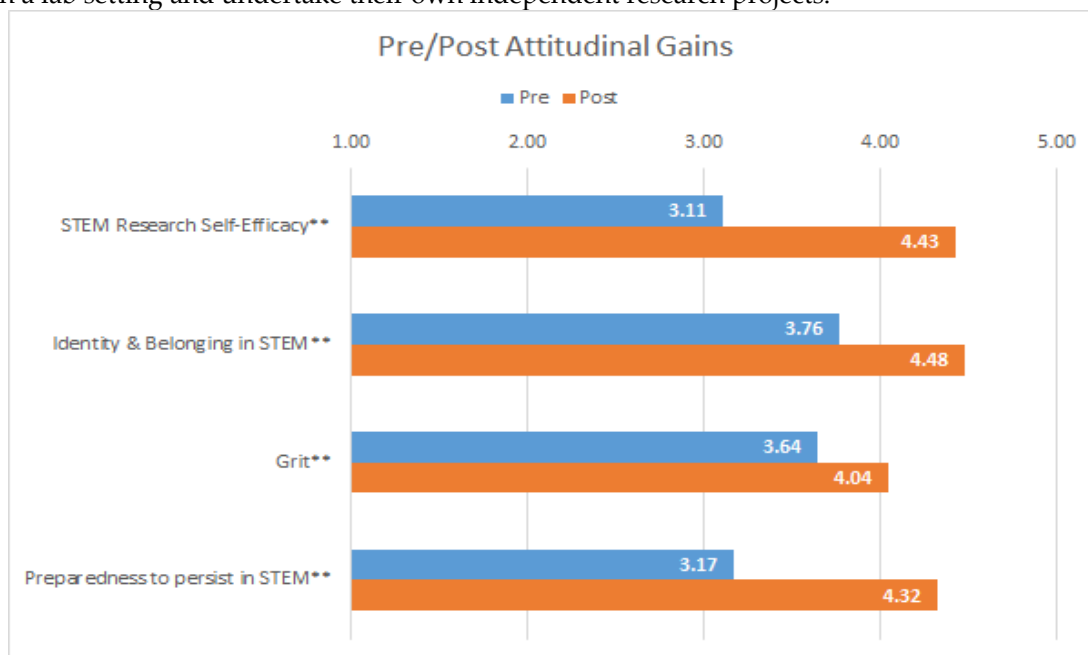
3.1 is a minimized variant of aptamer clone 4-3. The triplet GGG was added to 5' end to improve transcription efficiency and, thus CCC added to 3' end to participate in the stem structure, contributing to a lower dG.

### 3.2 Results of Student Assessment Outcomes

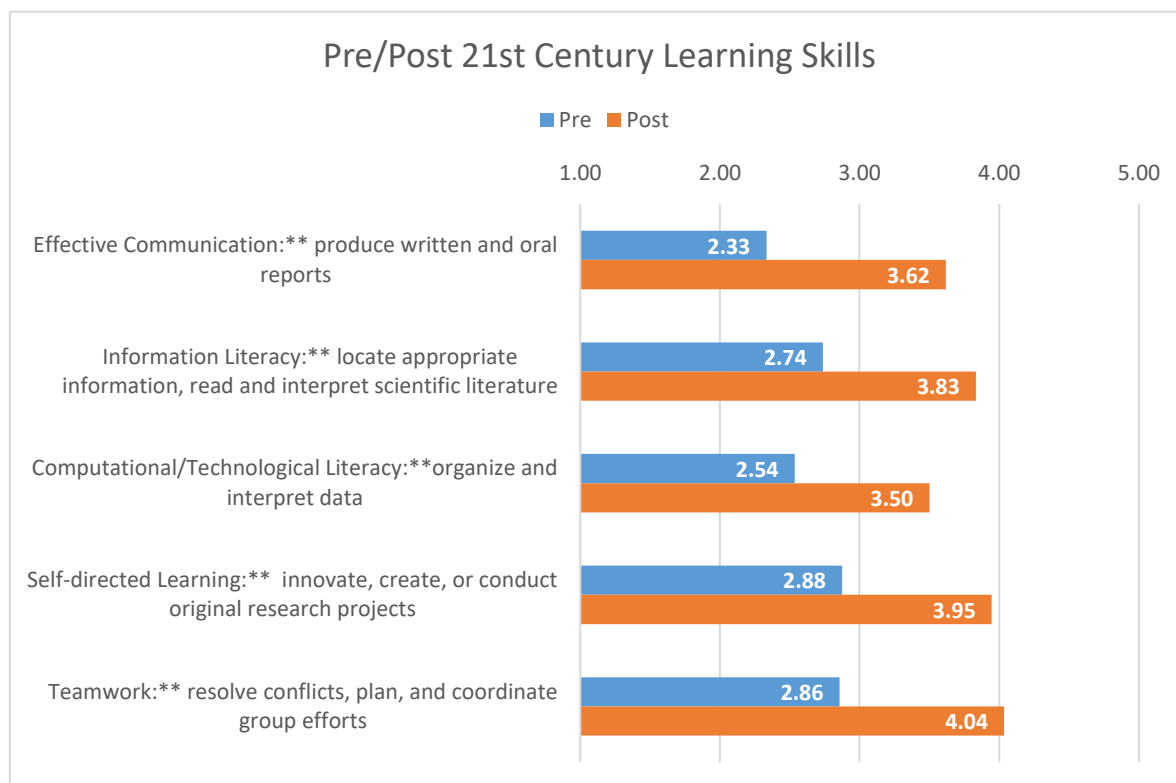
Using survey data collected in Fall 2017, paired samples t-tests were utilized to assess Aptamer Stream student gains from pre to post across attitudes (e.g., self-efficacy) and 21<sup>st</sup> Century Learning Skills (e.g., effective communication). Fourteen out of 24 aptamer students completed the pre/post student survey in Fall 2017 and consented to include their responses for research purposes. Given the small sample size, caution should be employed when interpreting the data as the findings displayed are considered preliminary only. Increasing the sample size and collecting data across multiple semesters is needed to validate and replicate these findings.

The findings, displayed in Figures 3 and 4, suggest that students express gains in both their attitudes and research skills from pre to post. Among students' attitudinal gains (Figure 3), the data suggest that students express statistically significant improvements in their research self-efficacy (e.g., "I have the skills to conduct my own STEM research project."), identity and belongingness (e.g., "I feel like I belong in STEM."), grit (e.g., "When I am working on a research problem that I can't immediately understand, I work harder to find a solution."), and preparedness to persist in STEM (e.g., "I feel prepared to do advanced coursework in STEM."). The largest gains were in research self-efficacy: before participating in the Aptamer Stream, students rated themselves a mean of 3.11 (Std.

dev: 0.83) on a 5-point Likert scale; after participating in the Aptamer Stream the mean increased to 4.43 (Std. dev: 0.53). Among their 21<sup>st</sup> Century Learning Skills (Figure 4), the data further suggest that students show statistically significant increases in their self-reported ability to communicate effectively (e.g., write scientific reports), locate and interpret information (e.g., read and interpret information in scientific literature), interpret and analyze data, engage in self-directed learning (e.g., conduct research independently), and work effectively on a team (e.g., communicate effectively with lab and research partners). Together, this provides preliminary evidence to suggest that the aptamer research experience provides students with the skills, confidence and know-how to conduct research within a lab setting and undertake their own independent research projects.



**Figure 3.** Pre/post attitudinal gains across 14 students participating in the Aptamer Stream in Fall 2017. Scale: 1, Strongly Disagree to 5, Strongly Agree. \*\* $p < .01$ , \* $p < .05$ . The data displayed above capture construct averages.



**Figure 4.** Pre/post research skill gains across 14 students participating in the Aptamer Stream in Fall 2017. Scale: 1, Poor to 5, Excellent. \*\* $p < .01$ , \* $p < .05$ . The data displayed above capture construct averages.

For formative purposes, the survey included a few open-ended questions and a satisfaction rating scale to further offer insights into students' general perceptions of the research experience. In terms of general satisfaction, 77% of students indicated that they would "very likely (5)" recommend participating in the aptamer experience to other students (scale: 1, very unlikely to 5, very likely). When asked to elaborate on their satisfaction rating, students indicated that they most valued and benefited from the hands-on learning experience of designing and executing their own research project. For example, one student said,

*"[This experience] allows us to truly delve into a research project of our own design, and forces us to think critically in a way no other lab can provide."*

Another student highlighted the lab skills that they learned in the stream and the uniqueness of this experience as a first-year STEM student:

*"I think that it is very valuable to learn lab techniques and skills such as gel electrophoresis or PCR as an undergraduate because we are exposed to research before we enter graduate school or upper division courses. Not many universities allow freshman/sophomores to be completing research in real labs so I think this is a very unique opportunity UT offers to the students."*

Overall, the preliminary survey data suggest that the Aptamer Stream provides students with an authentic hands-on learning environment whereby their attitudes and research skills are being cultivated and enhanced over time. While pre/post survey data provides some data to suggest that students express gains across key areas of development, it may not necessarily point to the efficacy of the aptamer stream in particular. That is, without a comparison group, the gains in attitudes and skills could be attributed to a maturation effect— a natural improvement in cognitive abilities that takes place during the college years. On-going education research comparing aptamer students to a matched comparison population are currently underway, and will further test the impact of this experience controlling for a possible maturation effect.

#### 4. Discussion

Integrating teaching and learning objectives into a research lab, the resulting Aptamer Stream lab yields positive outcomes in aptamer generation and student attitudes and research skills. This manuscript serves as a testament to positive research contributions, as well as the positive student outcomes.

Point in case, one of the early positive research outcomes was the identification and characterization of anti-CIAP RNA aptamers with nM affinities for CIAP. (Specifically, the anti-CIAP RNA aptamers 4-3, 4-9, 3-6, and minimized 3-1 variant have the following Kds, respectively: 5 nM, 9.4 nM, 10.8 nM, 6.7 nM.) After the one year CURE in the Aptamer Stream, undergraduate Vincent Huynh (co-author) identified anti-CIAP aptamers after nine rounds of *in vitro* aptamer selection. Through Huynh's remaining sophomore to senior year at The University, the anti-CIAP aptamers were studied, characterized, and used in the development of assays. While the assays development continues, Huynh's research has provided many notable contributions, including the establishment of radiation binding assay positive controls (i.e. the anti-CIAP aptamer and CIAP pair), development of teaching lab best practices and the sequence of aptamer analysis and characterization, as well the connection and development of a network of resources and expertise (e.g. Kd assay and analysis, activity assays, etc.). Furthermore, the anti-CIAP aptamers have the potential to be used in ELISA or ELONA assays, which, for example, could non-covalently connect the CIAP reporter molecule to a detection aptamer/antibody. Such an application could lead to forgoing the potentially costly chemical conjugation of detection aptamers or antibodies to instead use a simple, non-covalent reporter system.

None of these research contributions, however, would have been possible without the careful consideration of the environment, which included not only research priorities, but educational commitments. Briefly described here, this includes the consideration of the student, in general the students' *background knowledge, interest, timeframe*, as well as the minimal *budget, equipment, and materials* needs for a research course. Joining the Aptamer Stream in the second semester of their freshman year, most student researchers had little to no background in molecular biology or biochemistry (such as nucleic acid functionality, molecular biology techniques - PCR, gel electrophoresis, nucleic acid quantification, etc., assay development, etc.). However, student interest in the aptamer-field, especially in the area of generating medical diagnostics and therapeutics, in general is fairly high. This is most evident in that more students request this FRI stream/course than there are seats available and that the retention of students from the spring to the fall semester is high (>75% over multiple years). Additionally, in an effort to accommodate the undergraduate students' schedules, which are typically full of courses, student organizations (e.g. pre-medical/health organizations, mentoring organizations, honor societies, and organizations fundraising for a cause, etc.) and volunteering experiences, the lab operates approximately 9a to 6p M-F with undergraduate near-peer mentor support. This open-door policy provides the flexibility for students to conduct lab work within their own schedules.

With respect to minimizing equipment and materials expenses, this is an area fraught with creativity and innovation. Briefly, and for example, enzymes (such as *Taq* DNA polymerase and T7 RNA polymerase) are acquired through in-house preparations, collaborators, or even in the form of raw "cellular reagents" (i.e. lyophilized cell lysate containing overexpressed enzymes) [28]. While the cost of these enzymes are minimized, batch-to-batch activity variations introduces error and necessitates quality control measures, as well as an intentional focus on teaching such topics as "experimental controls" and "experimental design." To minimize equipment costs, cycle course PCR is utilized (in lieu of real-time PCR) to estimate the number of PCR cycles need to sufficiently amply selected pool. As a last example of cost-saving measures, new and more affordable materials (such as streptavidin-conjugated beads) are regularly tested and integrated into the lab.

Additional considerations in the development of research projects include the likelihood of project success. FRI streams are led by non-tenure track faculty members (Research Educators) with milder requirements to publish than tenure-track faculty, thus the streams have an opportunity to take on risky project with a small likelihood of success. Furthermore, aptamer selections using

canonical nucleotides have a small likelihood of success. SomaLogic reported < 30% of their aptamer selections against human proteins were successful when using RNA, DNA, or 2'-fluoro-pyrimidine RNA [29-30]. Further compounding the challenges of the research experience, the Aptamer Stream marks many students' first encounter with research. However, there may be some advantage to providing a challenging, yet attainable research goals, which, when met or nearly met, may promote positive attitudes towards science. In so much, some of the Aptamer Stream research projects were designed with this in mind, such as projects seeking the identification of *novel* aptamers against targets with known aptamers, thus validated aptamer targets (e.g. anti-lysozyme [31]), anti-CIAP [32], etc. aptamer projects). For example, CIAP has known aptamers [32], which predate Huynh's aptamers.

While the UT Aptamer Stream (est. 2006) was seemingly the first course-based undergraduate experience in aptamer research and served as an early model for such an experience, another aptamer CURE was recently established. The University of Maryland First-Year Innovation and Research Experience (FIRE) Engineering Biosensors Research Stream was launched in 2018. This undergraduate teaching and research lab specializes in "selecting and characterizing aptamers" and "designing and testing aptamer-based biosensors" [33]. Building a community of aptamer education, students in the FRI Aptamer Stream and the FIRE Engineering Biosensors Research Stream share a common blog, as well as peer review materials, in an effort to build collaboration and science communication skills. With the emergence of the FIRE Engineering Biosensors Research Stream and ideally new aptamer CURES, there is a potential for collaboration, sharing of resources and methodologies, and, most importantly, integrating students into the research experience.

In reflection on the Aptamer Stream to serve both research and educational objectives, a question about the "defining" features of the course are raised: Is there something about aptamer research that is intrinsically well-matched for an educational environment? Auchincloss et al. [34] proposed five essential features of CURES: scientific practices, iteration, discovery, relevance/broader impact, and collaboration. The iterative process of *in vitro* selection methodology, sieving of libraries in discovery-based research, broadly applicable aptamers (e.g. therapeutics, diagnostics, and sensor applications), and now the new multi-institutional Aptamers in Education community, which are all central to the work, speak to the natural fit of aptamers into the education realm of course-based undergraduate research experiences. This type of research experience is increasingly important for students to learn (from virtually their first days on campus) that they can manipulate biology themselves, and serves as a powerful springboard for a variety of professional experiences, from medical school to graduate school to direct entry into the biotechnology industry. Perhaps this new approach to education and aptamer research will open the doors to similar types of research experiences, benefitting both the aptamer community and undergraduate education as a whole.

**Supplementary Materials:** The following are available online at [www.mdpi.com/xxx/s1](http://www.mdpi.com/xxx/s1), Table S1: Anti-CIAP Aptamer Sequences, text file.

**Author Contributions:** Conceptualization, G.S. and A.E.; methodology, G.S., S.E., and A.E.; validation, G.S. and V.H.; formal analysis, G.S., S.E., and A.E.; investigation, S.E. and V.H.; resources, G.S., V.H., S.E., and A.E.; data curation, G.S. and S.E.; writing-original draft, G.S., V.H. and S.E.; writing-review and edition, G.S., V.H., S.E., and A.E.; visualization, G.S. and S.E.; supervision, G.S. and S.E.; project administration, G.S.; funding acquisition, G.S. and A.E.

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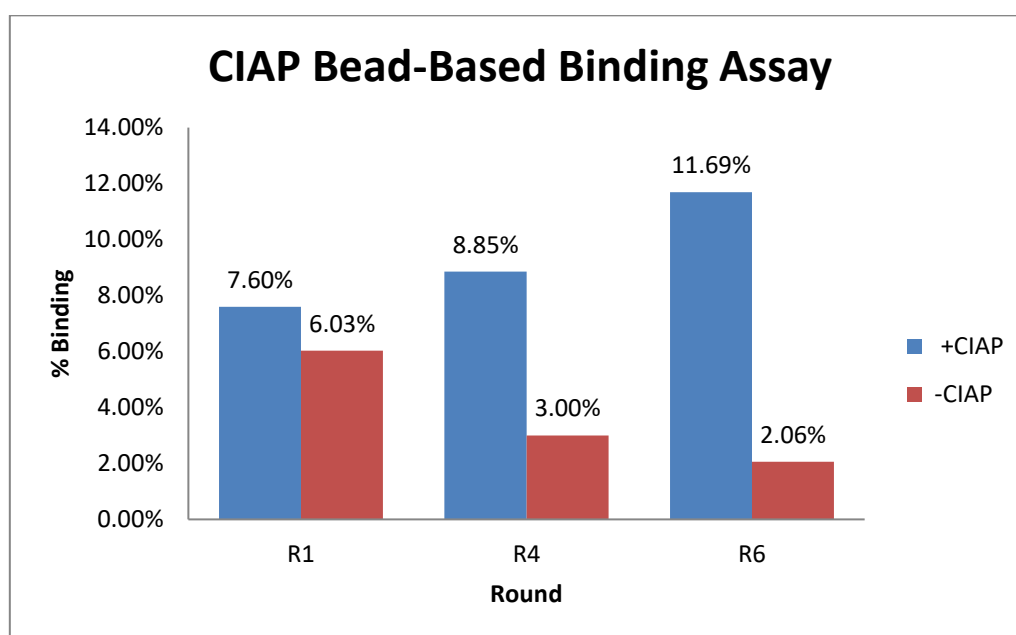
## Appendix A – CIAP Aptamer *in vitro* Selection Conditions

**Table 1.** CIAP aptamer *in vitro* selection conditions.

Round	RNA: CIAP (pmol : pmol)	Volume of Beads used (ul)	# of PCR cycles necessary to amplify selected pool	Total Wash volume (ml)	Amount of recovered pool (pmol)
Round 1	400pmol : 400pmol	50.0ul	10	2.4 mL	2093 pmol
Round 2*	400pmol : 400pmol	50.0ul	14	2.4 mL	388 pmol
Round 3*	300pmol : 300pmol	44.1ul	11	2.4 mL	301 pmol
Round 4*	120pmol : 100pmol	14.7ul	15	3.2 mL	969 pmol
Round 5	200pmol : 100pmol	14.7ul	9	3.2 mL	906 pmol
Round 6*	200pmol : 100pmol	14.7ul	9	4.0 mL	298 pmol
Round 7*	200pmol : 50pmol	7.35ul	9	6.0 mL	308 pmol
Round 8*	200pmol : 50pmol	7.35ul	13	13.9 mL	1241 pmol
Round 9*	800pmol : 50pmol	7.35ul	13	47.2 mL	735 pmol

\* A negative selection, exposing the selected RNA to the naked beads (i.e. beads without protein), was performed.

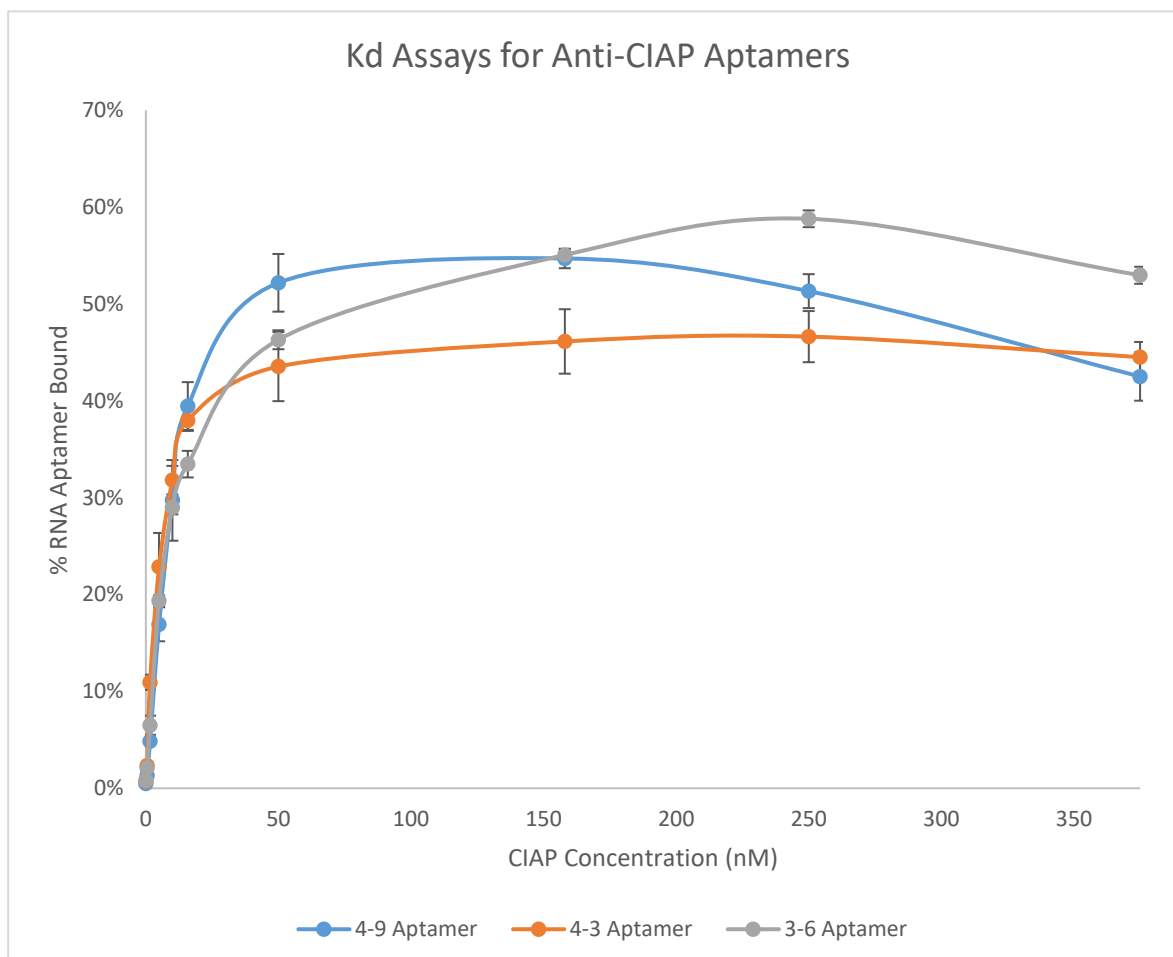
## Appendix B – Early anti-CIAP <sup>32</sup>P-labeled RNA binding assay results.



**Figure App. B.** Early anti-CIAP <sup>32</sup>P-labeled RNA binding assay results for round 1 (R1), round 4 (R4) and round 6 (R6) selected pools against biotinylated-CIAP on streptavidin beads. These early results

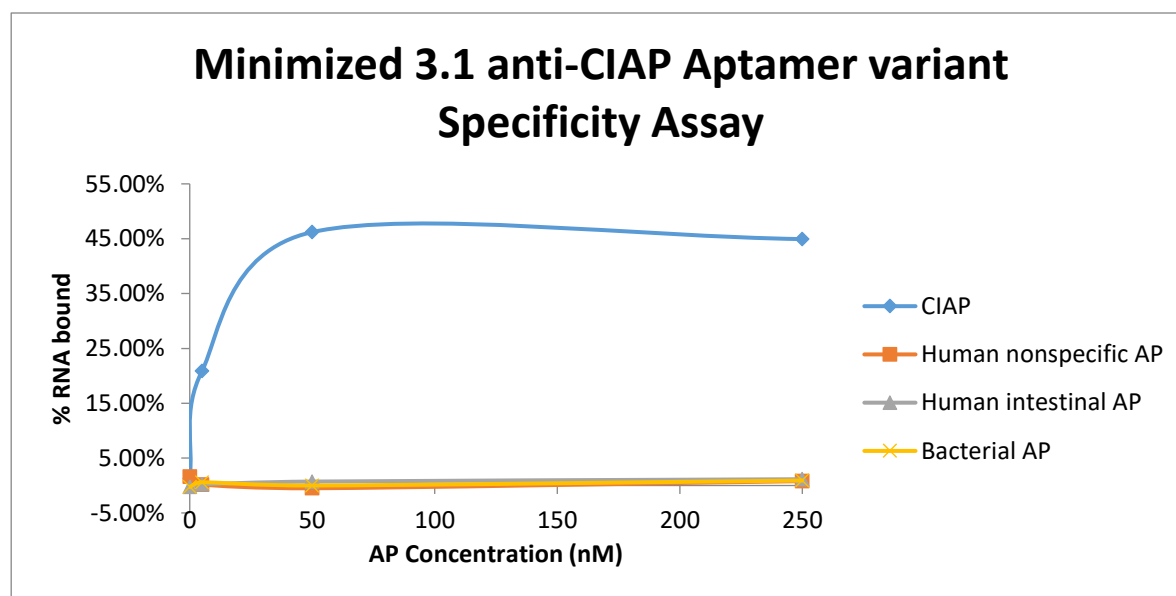
indicate an increase in selected RNA bound to CIAP over the rounds of selection and a decrease in the binding of the RNA pool bound to naked beads.

### Appendix C – Dissociation Constants (Kd) of anti-CIAP aptamers.



**Figure App. C.** Dissociation constants (Kd) of anti-CIAP aptamers. Kd assays were performed in triplicate using radioactive  $^{32}\text{P}$ -labeled RNA aptamers (5 nM). From these results, the anti-CIAP aptamers 4-3, 4-9, and 3-6 anti-CIAP aptamers were found to have the following Kds, respectively: 5 nM, 9.4 nM, and 10.8 nM.

## Appendix D – Aptamer Specificity Tests



**Figure App. D.** The target specificity assay involved the radiolabeled minimized 3.1 anti-CIAP aptamer variant and binding assays against a variety of alkaline phosphatases, including calf intestinal alkaline phosphatase (i.e. positive control), human tissue nonspecific alkaline phosphatase (recombinant), human intestinal alkaline phosphatase (recombinant), and bacterial alkaline phosphatase (recombinant). In this single point assay (i.e. no replicates), no significant aptamer binding was observed to the non-CIAP targets.

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