

The Arsenic-Binding Aptamer Cannot Bind Arsenic: Critical Evaluation of Aptamer Selection and Binding

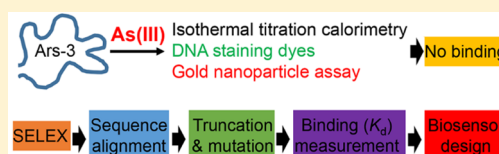
Chenghua Zong^{†,‡} and Juewen Liu^{*,‡}

[†]Department of Chemistry and Materials Science, Jiangsu Key Laboratory of Green Synthesis for Functional Materials, Jiangsu Normal University, Xuzhou, Jiangsu 221116, P.R. China

[‡]Department of Chemistry, Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

ABSTRACT: An arsenic-binding aptamer named Ars-3 was reported in 2009, and it has been used for detection of As(III) in more than two dozen papers. In this work, we performed extensive binding assays using isothermal titration calorimetry, various DNA-staining dyes, and gold nanoparticles. By carefully comparing Ars-3 and a few random control DNA sequences, no specific binding of As(III) was observed in each case.

Therefore, we conclude that Ars-3 cannot bind As(III). Possible reasons for some of the previously reported binding and detection were speculated to be related to the adsorption of As(III) onto gold surfaces, which were used in many related sensor designs, and As(III)/Au interactions were not considered before. The selection data in the original paper were then analyzed in terms of sequence alignment, secondary structure prediction, and dissociation constant measurement. These steps need rigorous testing before confirming specific binding of newly selected aptamers. This study calls for attention to the gap between aptamer selection and biosensor design, and the gap needs to be filled by careful binding assays to further the growth of the aptamer field.



Over the last two decades, aptamers have been extensively used in biosensor development.^{1–5} Aptamers are attractive because they are amenable to combinatorial selection under both physiological and nonphysiological conditions. At the same time, aptamers, especially DNA aptamers, have much better stability compared to antibodies. With excellent programmability and ease of modification, many signal transduction and signal amplification mechanisms have been demonstrated with a few model aptamers for analytes such as ATP,⁶ cocaine,⁷ thrombin,⁸ and Hg²⁺.⁹

The number of reported aptamers has exceeded 500 from the Aptagen online database. The lack of analytical work on many other aptamers could be due to a few reasons. For example, many aptamers are not well-characterized or not truncated, making it difficult for biosensor design. Sometimes, the target molecule is special and not available to most researchers.

An aptamer (named Ars-3) claimed to bind inorganic arsenic was reported in 2009 with high affinities for both As(III) ($K_d \approx 7$ nM) and As(V) ($K_d \approx 5$ nM).¹⁰ Arsenic is an important toxic element,^{11,12} and arsenic sensors have been extensively reported,¹³ using various nanomaterials,^{14–16} proteins,¹⁷ cells,¹⁸ and Raman spectroscopy.^{19,20} An arsenic aptamer would expand the range of recognition molecules. Indeed, Ars-3 has become quite popular, and the number of related papers for As(III) detection has reached at least 24 to date. Many of them used gold nanoparticles (AuNPs) and gold surfaces.^{21–31} The majority of these sensors detected only As(III), although As(V) was reported to have a similar binding affinity.

We recently studied the adsorption of As(III) by AuNPs and found that such adsorption was strong enough to inhibit DNA

adsorption.³² Such As(III)/AuNP interactions might cause false positive signals. Then, an important question is whether Ars-3 can specifically bind As(III) or if some of the previous observations were due to this artifact. We herein conducted a side-by-side comparison of Ars-3 versus a few random DNAs. Three independent methods were used to measure binding, including isothermal titration calorimetry (ITC), fluorescence spectroscopy, and the AuNP-based colorimetric method. No difference was observed between Ars-3 and the random sequences, and none of the DNA showed specific binding to arsenic.

We then went back to the original selection paper and rationalized possible reasons for the selection and measured As(III) binding. From aptamer selection to completion of biosensor development, there are many check points to ensure the analytical results, such as aptamer truncation and mutation, the use of random sequence DNAs as controls, independent methods for K_d measurement, and the choice of competing analytes. Some of these related to the Ars-3 are discussed here. The methods for critical evaluation of aptamer selection and binding are applicable for other aptamers.³³ We believe that filling the gap between aptamer selection and biosensor design is critical for the growth of the field.

MATERIALS AND METHODS

Chemicals. All DNA samples were from Integrated DNA Technologies (IDT, Coralville, IA). The sequences are listed in

Received: June 19, 2019

Accepted: July 25, 2019

Published: July 25, 2019

Table 1. $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$, NaAsO_2 , and other metal salts (NaF , NaCl , NaBr , NaI , Na_2SO_3 and $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, CaCl_2 , MgCl_2 , FeCl_2 , CuCl_2 , $\text{Pb}(\text{OAc})_2$, and CdCl_2) were from Sigma-Aldrich. 3-(N-Morpholino) propanesulfonic acid (MOPS) buffer was from Bio Basic Canada Inc. Citrate-capped AuNPs (13 nm) were prepared based on the method reported previously.³⁴ Milli-Q water was used for preparing buffers and solutions.

AuNP-Based Binding Assays. Three DNA sequences were compared: Ars-3, its complementary sequence (c-Ars-3), and a random sequence 30-mer DNA. For detection, 5 μL of a DNA (0.2 μM) was mixed with 29 μL of MOPS buffer (10 mM, pH 7.3), and then 20 μL of As(III) or As(V) was introduced. After incubation for 20 min at room temperature, 40 μL of the as-synthesized AuNPs were added, and the mixture was incubated for 40 min. Finally, 6 μL of NaCl (1 M) was added, and the absorption spectra were measured 10 min later using a spectrophotometer (Agilent 8453A). To test specificity, a few metal ions, including Ca^{2+} , Mg^{2+} , Fe^{2+} , Cu^{2+} , Pb^{2+} , and Cd^{2+} , and anions, including HAsO_4^{2-} , F^- , Cl^- , Br^- , I^- , SO_3^{2-} , and S^{2-} , were tested at 10 μM .

DNA-Staining Dye for Probing As(III) Binding. In a typical experiment, 5 μL of Ars-3 (2 μM) was mixed with 75 μL of HEPES (50 mM, pH 7.5, 150 mM NaCl, 0.5 mM MgCl_2 , 0.5 mM CaCl_2), and then 2 μL of As(III) of different concentrations was introduced. After 20 min of incubation, 20 μL of a dye solution (50 μM SG, 10 μM TO, or 10 μM ThT) was added, and the fluorescence spectra were collected by exciting at 485 nm for SG, 510 nm for TO, and 435 nm for ThT, respectively. For the Hg^{2+} positive control, the T₃₀ sequence was used with SG. For the G₁₅ positive control, 5 μL of G₁₅ (20 μM) was first mixed with 2 μL of K^+ (0.5 M), and then 20 μL of ThT (20 μM) was introduced.

ITC Measurements. ITC was conducted by using a VP-ITC microcalorimeter instrument (MicroCal). All the solutions were degassed to avoid air bubbles. The Ars-3 samples were dispersed in a MOPS buffer (10 mM, pH 7.3) with a final concentration of 10 μM and were loaded into the ITC cell (1.45 mL) at 25 °C. As(III) or As(V) (2 mM, 280 μL) in the same buffer was titrated (10 μL each time) into the cell through a syringe, except for the first injection which was 2 μL . As a positive control, As(III) was also titrated into a AuNP solution. The as-prepared AuNPs (10 nM, 13 nm) were centrifuged and washed twice (13000 rpm, 12 min) to remove the citrate added during the synthesis and redispersed with 10 mM MOPS (pH 7.3) buffer. Afterward, the sample was centrifuged at 3000 rpm for 4 min to remove the aggregated AuNPs, and the AuNPs were finally concentrated to 20 nM. The concentrated AuNPs were loaded into the ITC cell and were titrated with As(III) (2 mM, 280 μL).

RESULTS AND DISCUSSION

AuNP-Based Colorimetric Binding Assay. A frequently used method for aptamer-based sensing takes advantage of the colloidal stability and color change of AuNPs.^{35,36} Normally, adsorption of unfolded oligonucleotides (e.g., aptamers in the absence of their targets) can increase the stability of the AuNPs against salt to retain the red color (Figure 1A). Once an aptamer binds to its target to form a folded binding complex, its adsorption by AuNPs is hindered, and the AuNPs are easily aggregated by salt to give a blue color (Figure 1B). This strategy has been used for various aptamers,^{37–40} including Ars-3.^{24,25,31,41}

Table 1. DNA Sequences Used in This Work

DNA names	sequences (from 5' to 3')
Ars-3	GGTAATACGACTCACTATAGGGAGATACCAGCTTATTCAATTTTACAGAACCAACACGTCGCTCCGGGTACTTCTTCATCGAGATAGTAAGTCAATCT
c-Ars-3	AGATTGCACTTACTATCTCGATGAAGAAGTACCCGGAGCGACGTTGGTTCTGTAAATGAATAAGCTGGTATCTCCCTATAGTGAGTCGTATTACC
30-mer DNA	TTACGCACTCTGTGACAAACAAGGGGA
G ₁₅	GGGGGGGGGGGGG
T ₃₀	TTTTTTTTTTTTTTTTTTTTTTTTTTTTT

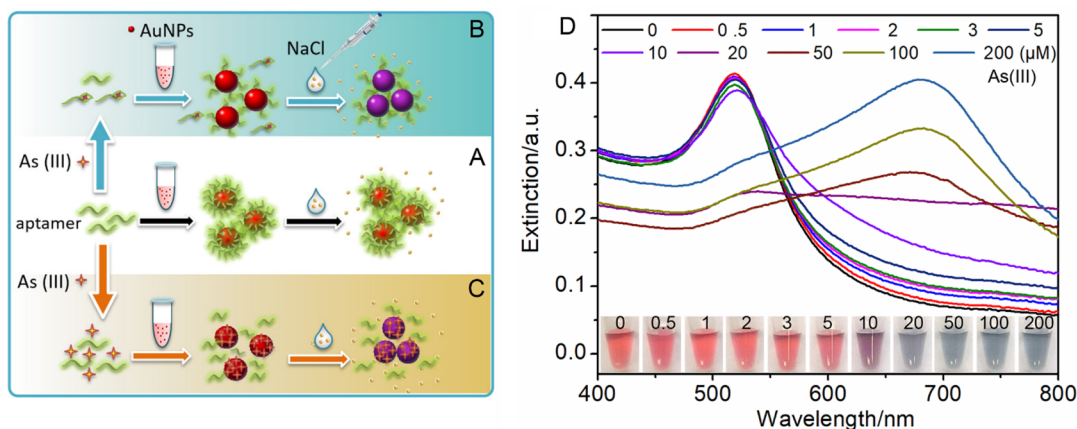


Figure 1. (A) Schemes of DNA adsorption on AuNPs increasing its colloidal stability against salt. The DNA can be an aptamer. Two different mechanisms explaining the color response to As(III): (B) aptamer–As(III) interaction induced folding of the aptamer and inhibited the adsorption of the aptamer by AuNPs, leading to the aggregation of AuNPs when treating with salts; (C) As(III) adsorption onto the AuNPs preventing the adsorption of the aptamer, leading to aggregation of the AuNPs by salt. (D) Photographs (inset) and UV–vis spectra of the 13 nm AuNPs after treating with increasing concentrations of As(III) (in μM) and NaCl in the presence of the Ars-3 aptamer.

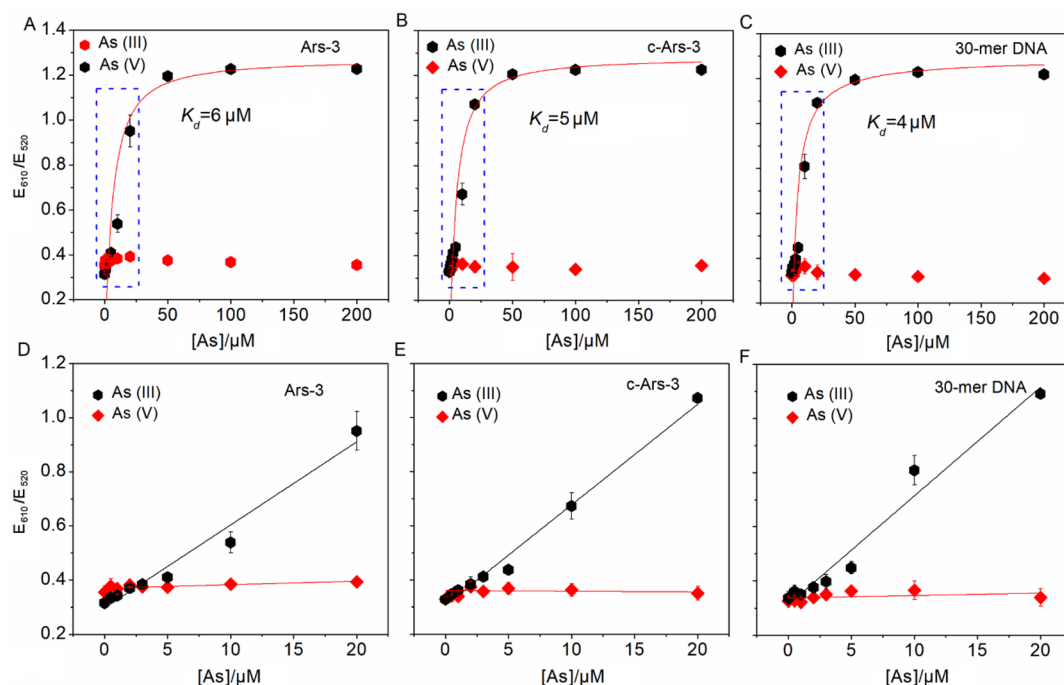


Figure 2. Sensitivity of (A) Ars-3, (B) c-Ars-3, and (C) a random 30-mer DNA for arsenic detection using the AuNP-based colorimetric assay. The As concentrations were 0, 0.5, 1, 2, 3, 5, 10, 20, 50, 100, and 200 μM . The corresponding linear responses in the range of 0–20 μM As in the boxes are plotted in panels D–F.

Most previous work studied only the originally reported full-length 100-mer Ars-3. To rigorously test aptamer binding, we also included two control sequences in this work: its complementary sequence (c-Ars-3, also 100-mer) and a random 30-mer DNA. Using the method described in Figure 1A,B and the Ars-3 sequence, we indeed observed the expected color change of the AuNPs from red to blue with increasing concentration of As(III) (inset of Figure 1D). The color change was quantified by UV–vis spectrometry (Figure 1D). With more As(III) added, the surface plasmon peak red-shifted. We used the ratio of extinction at 610 nm over 520 nm to quantify the color of the samples.

With the Ars-3 DNA, we observed saturated color change with 20 μM As(III), and no response was observed with

As(V), consistent with the literature reports.^{24,29} As low as 2 μM As(III) can be clearly distinguished from the blank (Figure 2A,D), and this is also in agreement with the numbers from a recent paper using this method after extensive optimization.³¹

However, the exact same trend was also observed with the c-Ars-3 DNA and the random 30-mer DNA (Figure 2B,C,E,F). Their apparent K_d 's (6 ± 1 , 5 ± 2 , and $4 \pm 1 \mu\text{M}$) and detection limits (1.7, 0.5, and 1.8 μM) were all very similar. Therefore, this claimed Ars-3 aptamer sequence did not show any advantage compared to the control DNAs. In addition, no obvious color change was observed for As(V) in all the studied samples, which is in conflict with the original Ars-3 selection paper claiming an even higher binding affinity for As(V).¹⁰ An alternative explanation to the color change is described in

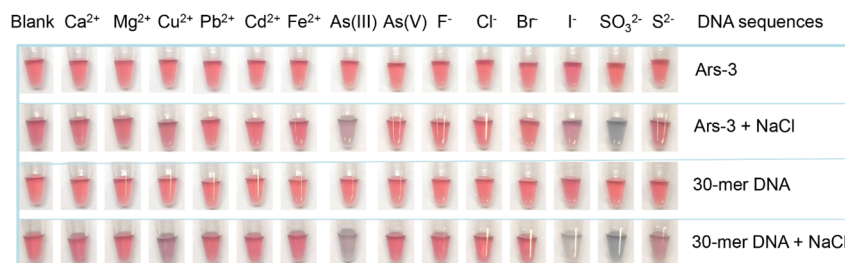


Figure 3. Colorimetric response of the Ars-3 and the 30-mer DNA toward different ions. The initial concentration of DNA was 10 nM with 4 nM 13 nm AuNPs dispersed in 10 mM MOPS buffer, pH 7.3. The concentration of the tested ions was 10 μ M.

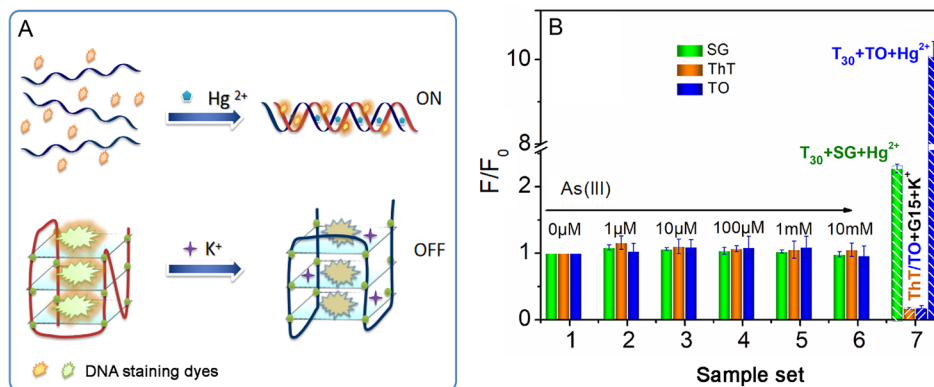


Figure 4. (A) Scheme showing using DNA-staining dyes to probe aptamer binding in two examples related to poly-T DNA binding Hg²⁺ and poly-G DNA binding K⁺. (B) Fluorescence intensity changes of the dye/aptamer systems when treated with increasing concentrations of As(III), where F_0 and F are the fluorescence intensities at the maximum emission wavelengths in the absence and presence of As(III), respectively. The final set of bars (set 7) are the positive controls with 10 μ M Hg²⁺ or 10 mM K⁺.

Figure 1C, where As(III) is adsorbed on the AuNPs to inhibit DNA adsorption. As(V), on the other hand, has much weaker affinity on gold and thus cannot inhibit DNA adsorption.³²

Although many As(III) detection papers used AuNPs, some of the assays did not involve gold. This needs to be analyzed carefully case by case. For example, the original paper also tethered the Ars-3 DNA to streptavidin agarose resin and demonstrated removal of both As(III) and As(V).¹⁰ Because appropriate controls such as the use of mutated DNA sequences are not available, it is difficult to rationalize this observation. Nevertheless, the high efficiency of removing both As(III) and As(V) suggested some nonspecific adsorption because Ars-3 was rarely reported to bind As(V) in most subsequent works.

Selectivity Test. To characterize a sensor, aside from sensitivity, another aspect is selectivity. As(III) exists as an anion in the form of AsO₃²⁻. Interestingly, most previous studies compared it with metal ions (cations), and the only other anion used was As(V) or HAsO₄²⁻. Here, we compared Ars-3 and the 30-mer DNA using both cations and anions (Figure 3). Among the tested cations, Cu²⁺ showed a purple color and other cations, including Ca²⁺, Mg²⁺, Pb²⁺, and Cd²⁺, remained red, consistent with previous reports.^{24,31} However, some anions, mainly I⁻, SO₃²⁻, and S²⁻, showed also a blue/purple color. Again, the model in Figure 1C can explain the effect of anions because I⁻, SO₃²⁻, and S²⁻ have strong affinities with AuNPs and they could also inhibit DNA adsorption.

Probing As(III) Binding Using DNA-Staining Dyes. Because the AuNP-based assay is complex and As(III) adsorption by the AuNPs can also cause the color change, we further tested binding using independent methods.

Normally, aptamer binding is accompanied by a conformational change, such as folding and formation of new base pairs,⁴² which can be probed by using DNA-staining dyes (Figure 4A). Typically, the fluorescence of such dyes can increase or decrease after aptamer binding to its target.^{43,44} To measure the interactions of Ars-3 and As(III), three dyes, including SG, ThT, and TO, were tested. SG fluorescence is enhanced upon binding to DNA duplex, and ThT is a common stain for G-quadruplex structures;⁴⁵ TO can stain both types of DNA.⁴⁶

Each dye was first mixed with Ars-3, and then As(III) was titrated. The fluorescence of the three dyes remained unchanged even with 10 mM As(III) (Figure 4B). For positive controls, we observed significant fluorescence enhancement when T₃₀ was mixed with SG or TO upon addition of Hg²⁺. In addition, when G₁₅ was mixed with TO or ThT, the fluorescence was quenched by K⁺. Because these dyes covered a range of DNA secondary structures, it suggests that As(III) might not be able to induce a specific folding of the DNA. Being an anion, As(III) is electrostatically repelled by DNA, and even nonspecific interactions might be low, which can explain why the fluorescence remained constant.

Measuring Binding Using ITC. The above optical methods are popular for sensing, but they do not directly measure binding. To further confirm binding, ITC was also used, which is a powerful technique for characterizing binding.⁴⁷ In the original paper, the reported K_d using surface plasmon resonance spectroscopy (SPR) was in the low nM region, and the K_d from the AuNP-based assay was around 6 μ M As(III). Within this affinity range, binding should be able to be accurately measured by ITC.

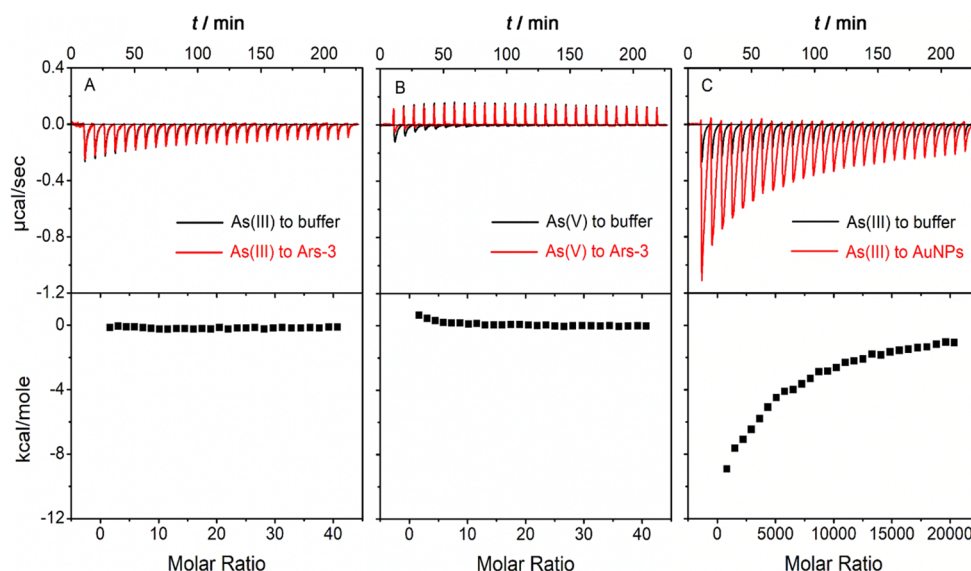


Figure 5. ITC traces of titrating (A) As(III) and (B) As(V) into 10 μ M Ars-3 and (C) titrating As(III) into 20 nM AuNPs in 10 mM MOPS buffer (pH 7.3). The original titration traces (top) and the integrated heat after background subtraction (bottom) are shown.

```

Ars-5  -----CAGTCAGA-----ATCCGGGCTTACACCCATTGTTTATTGTGCA-----
Ars-8  TGGGGATTGTACGTACACCACCAATTACACGCGAGTATG-----
Ars-3  -----TTACAGAACCAACGTCGCTCCGGGTACTTCTTCATCG-----
Ars-4  -----TTCCGCTAGGGGAACATGATCAACATGGACCAAGTAAAC
Ars-7  -----ATGC-----AAACC--CTTAAG--AAAGTGGTCGTCCAAAAACCATTG
Ars-6  ----CAATCTAAGC-----GAACCGCGTGCAGACCAATTACTCGCGCTA-----
Ars-1  -----ACCATCGCGGAAGTCCAGTCTGCCATCAAAATCCGAAGTG
Ars-2  -----CACGCGTTCAACCCCCGAATTAGCAATAGCAGATTACG

```

Figure 6. Alignment of the random region of the sequences from the original arsenic selection paper¹⁰ using the Clustal Omega software. The Ars-3 sequence is marked in red.

As(III) or As(V) was gradually titrated into the Ars-3 solution, and the amount of heat release was recorded as a function of time. We did not observe any heat for As(III) (Figure 5A) or As(V) (Figure 5B). As a control, As(III) was titrated into a dispersion of 13 nm AuNPs, and a significant heat release was observed, confirming the affinity of As(III) toward AuNPs.³² Therefore, ITC also failed to support specific aptamer binding of As(III). On the basis of the three independent methods for measuring binding, we conclude that the Ars-3 DNA is not an aptamer for As(III).

Gold Artifact. Given the lack of binding, why were many research groups able to detect As(III) with Ars-3? Interestingly, the original selection paper measured binding using SPR, which relied on a gold film. Many of the early biosensor designs used AuNPs. As described in Figure 1C, As(III) adsorption on gold can produce the exact same color response for colorimetric sensing, although the DNA does not need to bind As(III). The main controls used in those works were different metal ions for testing specificity, which could not discern this alternative binding mechanism either.

Gap between Aptamer Selection and Biosensor Design. This work has pointed out the lack of binding of the Ars-3 sequence. We hope this study can call for more careful control experiments should further studies be done with newly selected aptamer sequences. This work may have broader implications beyond this specific example. In the early days, most aptamers were carefully characterized biochemically including truncation and mutations to confirm binding. As the technique of systematic evolution of ligands by exponential

enrichment (SELEX) became widely adopted, more and more laboratories were able to carry out aptamer selection experiments. However, aptamer selection is prone to artifacts due to nonspecific binding. Without rigorous tests, false positive results might be produced (e.g., obtaining sequences that bind to the matrix for target immobilization or preferentially amplified by PCR). It appears that a gap exists between aptamer selection and biosensor design. This gap is the rigorous characterization of aptamers and confirming of binding. Aptamer binding assays are not very straightforward in many cases and are sometimes susceptible to artifacts.³³

Aptamer Sequence Analysis. We then went back to the original paper to see the source of problems at various check points. A total of eight DNA sequences were reported in the paper (named Ars-1 to Ars-8).¹⁰ We took the random region of the sequences and aligned them using Clustal Omega (Figure 6). The alignment result is poor, and it is hard to find two sequences with highly conserved regions. From their binding measurement, however, all these sequences had K_d between 5 nM and 100 nM for both As(III) and As(V). It is unlikely that so many different solutions exist for arsenic binding. The similar K_d for the distinct sequences could also be a flag of false positive binding measurements. At this point, had some random sequences been tested, potential artifacts might have been discovered.

Usually, successful aptamer selections should result in well-aligned sequences. The lack of a good sequence alignment also makes it difficult to make rational truncation and mutation studies. Nowadays, high throughput and deep sequencing

methods allow the trace of library enrichment in each round,⁴⁸ which can provide further information on enrichment of specific sequences. Secondary structure analysis was also performed in the original paper, but none of the sequences formed well-defined structures based on Mfold.⁴⁹ A few conserved hairpin loops were identified, but they were mainly in the primer binding regions instead of in the random region.

Truncation and Mutations. The Ars-3 sequence is 100 nucleotides long, including 60 from the fixed regions and 40 from the random region. Given what is currently known, for a small target like As(III) or As(V), its aptamer is unlikely to need such a long sequence, especially for aptamers obtained by SELEX. For example, Hg²⁺ and Ag⁺ can be bound by two pyrimidine bases.⁵⁰ Short G-quadruplex DNA can bind K⁺, and the key nucleotides for a Na⁺ binding aptamer are below 30 nucleotides.^{51–53} A few more complex metal-binding aptamers were reported, and their lengths were all below 63 nucleotides.^{54–56} Although some very long aptamers are found in riboswitch, they can usually fold into well-defined structures,^{57,58} but this is not the case for the Ars-3 sequence. To date, however, no published efforts have been made to truncate Ars-3, and all used the full-length sequence. Truncation is a good way to confirm binding and to optimize sensor design.

Mutation of critical nucleotides is another important step to ensure binding. For example, mutating one or two critical nucleotides can fully inhibit binding of ATP,⁵⁹ Na⁺,^{52,53} and cocaine⁶⁰ by their respective aptamers. If every nucleotide can be mutated, it is an indication of nonspecific binding. In this work, we used the cDNA of the Ars-3 as control, and it can be considered as a random sequence. Using a random sequence of the same length can also flag false positive binding.

K_d Measurement and Specificity Test. For aptamer characterization, K_d determination is a critical step. This is not easy in many cases, and artifacts can arise. For example, the same aptamer can vary its K_d over a few orders of magnitude just by using different measurement methods.³³ In the original paper, the authors performed extensive K_d measurements using SPR, which measures the change of dielectric constant near the gold surface induced by binding. Their measurement, however, did not consider As(III)/gold interactions, or the nonspecific DNA adsorption onto the gold surface. A related assay is binding specificity. As mentioned above, most papers only compared As(III) with cations instead of anions. Problems might be identified if anions such as I[−], SO₃^{2−}, or S^{2−} were used.

Chemical Feasibility of Arsenic Binding by DNA. Now that we argued against Ars-3 being a real aptamer, whether aptamers for As(III) and As(V) exist remains an open question. As(V) is known to be chemically similar to phosphate, and it is likely repelled by DNA (just like phosphate is repelled by DNA). The chance of obtaining aptamers for As(V) might be low. The similarity between As(V) and phosphate, however, could be useful for developing competitive assays.^{14,15,61,62} As(III) is a softer ligand, and it has strong affinity with thiol. The interactions between arsenic and DNA are mainly reported in the context of inducing DNA damage.^{63,64} If As(III) and DNA bases can interact, selection of aptamers for it may indeed still be possible.

CONCLUSIONS

In summary, we measured binding of the Ars-3 sequence to As(III) using three independent methods, and comparisons

were made with a few control DNA sequences. None of the measurements showed any indication of specific As(III) binding. We also rationalized a possible reason for the previously observed binding due to adsorption of As(III) by gold surfaces. We further analyzed the original aptamer selection paper and discussed the various check points from sequence alignment, secondary structure analysis, truncation and mutation, K_d measurement, and test of competing analytes. Rigorous work on the biochemical side is crucial for newly selected aptamers to confirm specific binding. We hope this work can call attention to the gap between aptamer selection and biosensor design and fuel further growth of the aptamer field.

AUTHOR INFORMATION

Corresponding Author

*E-mail: liujw@uwaterloo.ca.

ORCID

Juewen Liu: 0000-0001-5918-9336

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Funding for this work was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC), Canada First Research Excellence Fund (CFREF) for the Global Water Future project, the National Natural Science Foundation of China (21804060), Science and Technology Innovation Project of Xuzhou (KC 18137). C.Z. was supported by the Jiangsu overseas visiting scholar program to visit the University of Waterloo.

REFERENCES

- (1) Famulok, M.; Hartig, J. S.; Mayer, G. *Chem. Rev.* **2007**, *107*, 3715–3743.
- (2) Zhou, W.; Saran, R.; Liu, J. *Chem. Rev.* **2017**, *117*, 8272–8325.
- (3) Liu, J.; Cao, Z.; Lu, Y. *Chem. Rev.* **2009**, *109*, 1948–1998.
- (4) Zhao, W.; Brook, M. A.; Li, Y. *ChemBioChem* **2008**, *9*, 2363–2371.
- (5) Fang, X. H.; Tan, W. H. *Acc. Chem. Res.* **2010**, *43*, 48–57.
- (6) Huizenga, D. E.; Szostak, J. W. *Biochemistry* **1995**, *34*, 656–665.
- (7) Stojanovic, M. N.; de Prada, P.; Landry, D. W. *J. Am. Chem. Soc.* **2001**, *123*, 4928–4931.
- (8) Bock, L. C.; Griffin, L. C.; Latham, J. A.; Vermaas, E. H.; Toole, J. J. *Nature* **1992**, *355*, 564–566.
- (9) Ono, A.; Togashi, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 4300–4302.
- (10) Kim, M.; Um, H. J.; Bang, S.; Lee, S. H.; Oh, S. J.; Han, J. H.; Kim, K. W.; Min, J.; Kim, Y. H. *Environ. Sci. Technol.* **2009**, *43*, 9335–9340.
- (11) Shen, S.; Li, X.-F.; Cullen, W. R.; Weinfeld, M.; Le, X. C. *Chem. Rev.* **2013**, *113*, 7769–7792.
- (12) Nordstrom, D. K. *Science* **2002**, *296*, 2143–2145.
- (13) Yogarajah, N.; Tsai, S. S. H. *Environ. Sci.: Water Res. Technol.* **2015**, *1*, 426–447.
- (14) Liu, B.; Liu, J. *Chem. Commun.* **2014**, *50*, 8568–8570.
- (15) Lopez, A.; Zhang, Y.; Liu, J. *J. Colloid Interface Sci.* **2017**, *493*, 249–256.
- (16) Xia, N.; Shi, Y.; Zhang, R.; Zhao, F.; Liu, F.; Liu, L. *Anal. Methods* **2012**, *4*, 3937–3941.
- (17) Liu, Y.; Yu, C.; Cao, Z.; Shvarts, D.; Trogler, W. C. *Chem. - Eur. J.* **2017**, *23*, 10289–10292.
- (18) Merulla, D.; Buffi, N.; Beggah, S.; Truffer, F.; Geiser, M.; Renaud, P.; van der Meer, J. R. *Curr. Opin. Biotechnol.* **2013**, *24*, 534–541.

- (19) Mulvihill, M.; Tao, A.; Benjauthrit, K.; Arnold, J.; Yang, P. *Angew. Chem., Int. Ed.* **2008**, *47*, 6456–6460.
- (20) Li, J.; Chen, L.; Lou, T.; Wang, Y. *ACS Appl. Mater. Interfaces* **2011**, *3*, 3936–3941.
- (21) Yuan, M.; Zhang, Q. Q.; Song, Z. H.; Ye, T.; Yu, J. S.; Cao, H.; Xu, F. *Microchim. Acta* **2019**, *186* DOI: 10.1007/s00604-019-3373-1.
- (22) Vega-Figueroa, K.; Santillan, J.; Ortiz-Gomez, V.; Ortiz-Quiles, E. O.; Quinones-Colon, B. A.; Castilla-Casadiegos, D. A.; Almodovar, J.; Bayro, M. J.; Rodriguez-Martinez, J. A.; Nicolau, E. *ACS Omega* **2018**, *3*, 1437–1444.
- (23) Ensafi, A. A.; Akbarian, F.; Heydari-Soureshjani, E.; Rezaei, B. *Biosens. Bioelectron.* **2018**, *122*, 25–31.
- (24) Zhan, S. S.; Yu, M. L.; Lv, J.; Wang, L. M.; Zhou, P. *Aust. J. Chem.* **2014**, *67*, 813–818.
- (25) Siddiqui, M. F.; Kim, S.; Jeon, H.; Kim, T.; Joo, C.; Park, S. *Sensors* **2018**, *18*, 777.
- (26) Thao Nguyen, N. L.; Park, C. Y.; Park, J. P.; Kailasa, S. K.; Park, T. J. *New J. Chem.* **2018**, *42*, 11530–11538.
- (27) Song, L. L.; Mao, K.; Zhou, X. D.; Hu, J. M. *Talanta* **2016**, *146*, 285–290.
- (28) Yang, B.; Chen, X.; Liu, R.; Liu, B.; Jiang, C. *RSC Adv.* **2015**, *5*, 77755–77759.
- (29) Wu, Y. G.; Zhan, S. S.; Wang, F. Z.; He, L.; Zhi, W. T.; Zhou, P. *Chem. Commun.* **2012**, *48*, 4459–4461.
- (30) Wu, Y.; Liu, L.; Zhan, S.; Wang, F.; Zhou, P. *Analyst* **2012**, *137*, 4171–4178.
- (31) Matsunaga, K.; Okuyama, Y.; Hirano, R.; Okabe, S.; Takahashi, M.; Satoh, H. *Chemosphere* **2019**, *224*, 538–543.
- (32) Zong, C.; Zhang, Z.; Liu, B.; Liu, J. *Langmuir* **2019**, *35*, 7304–7311.
- (33) McKeague, M.; De Girolamo, A.; Valenzano, S.; Pascale, M.; Ruscito, A.; Velu, R.; Frost, N. R.; Hill, K.; Smith, M.; McConnell, E. M.; DeRosa, M. C. *Anal. Chem.* **2015**, *87*, 8608–8612.
- (34) Liu, J.; Lu, Y. *Nat. Protoc.* **2006**, *1*, 246–252.
- (35) Li, H.; Rothberg, L. J. *J. Am. Chem. Soc.* **2004**, *126*, 10958–10961.
- (36) Li, H.; Rothberg, L. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 14036–14039.
- (37) Zhang, J.; Wang, L. H.; Pan, D.; Song, S. P.; Boey, F. Y. C.; Zhang, H.; Fan, C. H. *Small* **2008**, *4*, 1196–1200.
- (38) Wei, H.; Li, B.; Li, J.; Wang, E.; Dong, S. *Chem. Commun.* **2007**, 3735–3737.
- (39) Lee, J. H.; Wang, Z.; Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2008**, *130*, 14217–14226.
- (40) Hoang, M.; Huang, P.-J. J.; Liu, J. *ACS Sensors* **2016**, *1*, 137–143.
- (41) Tang, M.; Wen, G.; Liang, A.; Jiang, Z. *Luminescence* **2014**, *29*, 603–608.
- (42) Hermann, T.; Patel, D. J. *Science* **2000**, *287*, 820–825.
- (43) Stojanovic, M. N.; Landry, D. W. *J. Am. Chem. Soc.* **2002**, *124*, 9678–9679.
- (44) Wang, J.; Liu, B. *Chem. Commun.* **2008**, 4759–4761.
- (45) Yeasmin Khusbu, F.; Zhou, X.; Chen, H.; Ma, C.; Wang, K. *TrAC, Trends Anal. Chem.* **2018**, *109*, 1–18.
- (46) Lubitz, I.; Zikich, D.; Kotlyar, A. *Biochemistry* **2010**, *49*, 3567–3574.
- (47) Zhang, Z.; Zhang, X.; Liu, B.; Liu, J. *J. Am. Chem. Soc.* **2017**, *139*, 5412–5419.
- (48) Schütze, T.; Wilhelm, B.; Greiner, N.; Braun, H.; Peter, F.; Mörl, M.; Erdmann, V. A.; Lehrach, H.; Konthur, Z.; Menger, M.; Arndt, P. F.; Glökler, J. *PLoS One* **2011**, *6*, No. e29604.
- (49) Zuker, M. *Nucleic Acids Res.* **2003**, *31*, 3406–3415.
- (50) Ono, A.; Torigoe, H.; Tanaka, Y.; Okamoto, I. *Chem. Soc. Rev.* **2011**, *40*, 5855–5866.
- (51) Torabi, S.-F.; Wu, P.; McGhee, C. E.; Chen, L.; Hwang, K.; Zheng, N.; Cheng, J.; Lu, Y. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 5903–5908.
- (52) Zhou, W.; Ding, J.; Liu, J. *Nucleic Acids Res.* **2016**, *44*, 10377–10385.
- (53) Zhou, W.; Zhang, Y.; Huang, P.-J. J.; Ding, J.; Liu, J. *Nucleic Acids Res.* **2016**, *44*, 354–363.
- (54) Wang, H.; Cheng, H.; Wang, J.; Xu, L.; Chen, H.; Pei, R. *Talanta* **2016**, *154*, 498–503.
- (55) Rajendran, M.; Ellington, A. D. *Anal. Bioanal. Chem.* **2008**, *390*, 1067–1075.
- (56) Chen, Y.; Li, H.; Gao, T.; Zhang, T.; Xu, L.; Wang, B.; Wang, J.; Pei, R. *Sens. Actuators, B* **2018**, *254*, 214–221.
- (57) Winkler, W. C.; Breaker, R. R. *Annu. Rev. Microbiol.* **2005**, *59*, 487–517.
- (58) Furukawa, K.; Ramesh, A.; Zhou, Z.; Weinberg, Z.; Vallery, T.; Winkler, W. C.; Breaker, R. R. *Mol. Cell* **2015**, *57*, 1088–1098.
- (59) Dieckmann, T.; Butcher, S. E.; Sassanfar, M.; Szostak, J. W.; Feigon, J. *J. Mol. Biol.* **1997**, *273*, 467–478.
- (60) Neves, M. A. D.; Reinstein, O.; Saad, M.; Johnson, P. E. *Biophys. Chem.* **2010**, *153*, 9–16.
- (61) Wang, X.; Lopez, A.; Liu, J. *Langmuir* **2018**, *34*, 7899–7905.
- (62) Wang, X.; Liu, B.; Liu, J. *Langmuir* **2018**, *34*, 15871–15877.
- (63) Nafisi, S.; Sobhanmanesh, A.; Alimoghaddam, K.; Ghavamzadeh, A.; Riahi, H.-A. T. *DNA Cell Biol.* **2005**, *24*, 634–640.
- (64) Lee-Chen, S. F.; Gurr, J. R.; Lin, I. B.; Jan, K. Y. *Mutat. Res., DNA Repair* **1993**, *294*, 21–28.