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SUPPLEMENTARY INFORMATION

Isolation of DNA aptamers for herbicides under varying divalent metal ion concentrations

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SUPPLEMENTAL MATERIALS AND METHODS

Oligonucleotides

DNA oligonucleotides were purchased from Integrated DNA Technologies (IDT, Coralville, IA). Prior to use, oligos were purified by denaturing polyacrylamide gel electrophoresis (PAGE) using 1X TBE running buffer (89mM each of Tris and boric acid, 2mM EDTA, pH 8.3). Samples were extracted from the gel using TEN buffer (10mM Tris, pH 8.0, 1mM EDTA, 300mM NaCl), recovered by ethanol precipitation, and quantified by UV absorbance. The single-stranded DNA (ssDNA) random pool for in vitro selection was 5'-GAACTAGATCGCAGC-N_{20}-CGGCAGTGACTCTTGAAC-N_{20}-GGATCGAGGTAATCC-3'. The N_{20} designations indicate 20 nucleotides with equimolar incorporation of the four DNA bases at each position. The capture oligo that was complementary to the internal fixed sequence of the DNA pool and was used to capture the DNA pool during selection and subsequent testing was 5'-NH_2-CAACAACAACAACAACAAGAATCGCTGCCG-3'. Sequences of the isolated aptamers are provided in Table S1.

Reagents

MagnaBind carboxyl-derivatized beads and magnetic separators were purchased from Thermo Scientific (Rockford, IL). Atrazine was purchased from Cayman Chemical (Ann Arbor, MI), alachlor and metolachlor were purchased from Ultra Scientific (N. Kingstown, RI), and simazine was purchased from Alfa Aesar (Ward Hill, MA). Each herbicide was suspended in ethanol for use. Corning DNA-Bind Surface 96-well plates were purchased from Sigma Aldrich (St. Louis, MO).

Derivatization of magnetic beads with capture oligo

The 5' amine-modified capture oligo was coupled to MagnaBind carboxyl-derivatized beads using 50mM 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) in 100mM MOPS pH 7.0 and 1M NaCl. Reaction were incubated at room temperature for 10min with gentle agitation. The amount of capture oligo coupled to the beads was determined spectroscopically by measuring and comparing the A_{260} and A_{280} of the coupling solution before and after the coupling reaction.

Pre-selection

300pmol of random pool was annealed in 1x HEPES (5mM HEPES pH 7.5, 10mM NaCl, and 0.1mM EDTA) annealing buffer by heating at 95°C for 3min, followed by cooling on ice for 5min. The annealed pool sequences were incubated with carboxyl-derivatized magnetic beads lacking the capture oligo for 45min at room temperature. Separation of bound and unbound sequences was achieved using a magnetic separator to attract the derivatized beads to the side of the reaction tube and unbound pool oligos in solution were removed by

pipetting. Beads were then washed with appropriate binding buffer without the target herbicide, followed by two washes with 1X HEPES annealing buffer. The recovered pool sequences were then used to initiate the selections.

***In vitro* selection**

Magnetic beads were functionalized with the capture oligo as described above and were washed twice with 1X HEPES annealing buffer prior to use. For each round, the DNA pool was annealed by heating to 95°C for 3min, followed by incubation on ice for 5min in 1X HEPES annealing buffer prior to use in the selection step. After the pre-selection step, the pool sequences that did not bind to non-functionalized beads were used to initiate the selection by incubating with 100pmol capture oligo on derivatized beads for 1hr at room temperature. After removal of the unbound sequences, the bead-bound pool oligos were incubated with either atrazine or alachlor under one of the three selection conditions listed in the main text for 15min, followed by a second incubation of 45min. Separation was achieved using a magnetic separator and the eluted DNA in solution was removed by pipetting. Beads were then washed with binding buffer without the target herbicide, followed by two washes with 1X HEPES annealing buffer.

In the initial round of selection, the eluted DNA was collected and amplified via PCR using α-^{32}P-dCTP to incorporate a radiolabel as described below. In subsequent selection rounds, the amount of DNA present in the target elution fractions, post-elution washes, and remaining with the beads was determined by Cerenkov counting using a Tri-Carb 3110 TR (Perkin Elmer), as described previously (Emahi et al, 2015). The percentage eluted was determined from radioactivity in the combined elution washes based on the following equation: % eluted = [cpm in elution washes / sum of cpm in all washes (target elution, post-washing, and reconstituted beads)] x 100. Selection progression was monitored by determining the percent of radiolabeled DNA that eluted from the beads. Once binding activity was established in each selection, the resulting aptamers were cloned and sequenced as described below. Unique sequences were made via solid phase synthesis by IDT and are listed in Table S1.

PCR amplification

During PCR amplification, the two primers, primer 1: 5'-CAACAACAACAA-X-GGATTACCTCGATCC-3' and primer 2: 5'-GAACTAGATCGCAGC-3', anneal to the 3' and 5' ends of the randomized pool, respectively. Primer 1 contains a non-amplifiable spacer 18 linker (designated as X) that allows for separation of the inactive complement from the active pool sequence via PAGE following PCR amplification. The binding sequences eluted during the selection step were concentrated using an Amicon Ultra-0.5 Centrifugal Filter Device (MilliPore, Billerica, MA) and amplified via PCR for the next selection round. Ten cycles of PCR were performed in 1X Taq buffer (20mM Tris pH 8.8,

10mM KCl, 10mM $(NH_4)SO_4$, and 0.1% Triton X-100), 1mM dNTPs, 1.5mM $MgSO_4$, 0.5µM Primer 1 and 2µM Primer 2, and lab-prepared Taq polymerase. After a denaturation step of 95°C, reactions were cycled 10 times at 95°C for 30sec, 55°C for 30sec, and 72°C for 20sec. The resulting PCR products were extracted with an equal volume of 25:24:1 phenol:chloroform:isoamyl alcohol (PCI), followed by a second extraction with an equal volume of 24:1 chloroform:isoamyl alcohol (CI). 2µL of the extracted 10 Cycle PCR products was used as the template for 30 cycles of PCR. Reaction conditions were the same as 10 Cycle PCR, except the primer concentrations were changed to 1.25µM and 5µM, respectively, and α-^{32}P-dCTP was included in the reaction to label the resulting DNA strands with a radioactive tracer. The number of cycles was also increased from 10 to 30. The resulting products were purified via denaturing PAGE. The non-amplifiable spacer in Primer 1 allows for separation of the inactive compliment from the active pool sequence. The desired DNA strands were excised from the gel and isolated as described above, before being carried forward to the next round of selection.

Cloning and sequencing

Once binding activity was observed over several rounds for a selection, the isolated DNA oligonucleotides were cloned and sequenced to determine the resulting aptamers. The eluted DNA from round 7 of selections BC, BE, BG, and BJ and round 8 of selections BL and BN was amplified via PCR as before, but without α-32P-dCTP and using two different PCR primers to generate a double-stranded product. The new primers, 5'-TAATTAATTAATTAGGATTACCTCGATCC-3' and 5'-TAATTAATTAATTAGAACTAGATCGCAGC-3', anneal to the 3' and 5' ends of the DNA pool, respectively. The underlined portions of the primers introduce stop codons into the amplified products to reduce false negative in subsequent blue-white screening (Langner and Klussmann, 2003). The resulting PCR products were then cloned using the TOPO TA Cloning Kit purchased from Invitrogen (Carlsbad, CA). Plasmids from the resulting colonies were isolated via miniprep (IBI Scientific, Peosta, IA). Plasmids that were determined to have aptamer inserts were sequenced by the Protein and Nucleic Acid Chemistry Laboratory at Washington University (St Louis, MO). For the BC selections, 11 unique sequences were identified (7BC6, 7BC7, and 7BC8b were found twice) from 14 clones. Of 12 BE clones, 10 were unique (7BE2 and 7BE12 were found twice). For the BG selections, 6 unique sequences were found in 13 clones, with 7BG1 being duplicated and 7BG2 being found seven times. Selections BJ and BL yielded fewer clones. Four unique sequences were identified from 8 BJ clones, with 7BJ9 being duplicated and 7BJ1 being found 4 times. Just 2 unique sequences were found from the 6 BL clones (2 of 8BL13 and 4 of 8BL3). The 16 BN clones produced 10 unique sequences, with 8BN10, 8BN13, and 8BN27 appearing 3 times each. Unique sequences were made via solid phase synthesis by IDT and are listed in Table S1.

Fluorescent labeling of oligonucleotides

Oligonucleotides for testing were 5' phosphorylated using T4 polynucleotide kinase and ATP or were ordered with a 5'- amine modification for IDT. For phosphorylated oligonucleotides, 10µM oligo was incubated with 120mM imidazole pH 9, 50mM diethylenediamine, 151mM DMT-MM, and 5mM appropriate NHS ester (either TAMRA or fluorescein) in 50% (v/v) DMSO at 37°C for 3 – 4hr with occasional agitation. 5' amine modified oligos were labeled with either TAMRA or fluorescein NHS ester as previously described (Baum and Silverman, 2007). Following the labeling reactions, oligonucleotides were ethanol precipitated and purified via PAGE as described above.

Plate preparation

Prior to coupling, Corning DNA-Bind Surface plates were washed three times with autoclaved ultrapure (18MΩ) water for 5min and the washes were quantified. Next, 20pmol of the capture oligo was coupled to each well of the plates using 50mM DMT-MM in 100mM MOPS pH 7.0 and 1M NaCl for 12hr at 4°C. Unattached capture oligo was collected for quantification. Following coupling, plates were washed three times with autoclaved 18MΩ water for 5min and the washes were quantified. Unreacted NOS functional groups remaining in the wells were blocked using a 50mM Tris pH 7.5 wash for 15min. Remaining buffer was removed by washing the plates three times with autoclaved 18MΩ water for 5min. The amount of anchor oligo coupled to the plates was determined spectroscopically by measuring and comparing the A_{260} and A_{280} of the coupling solution before and after the coupling reaction.

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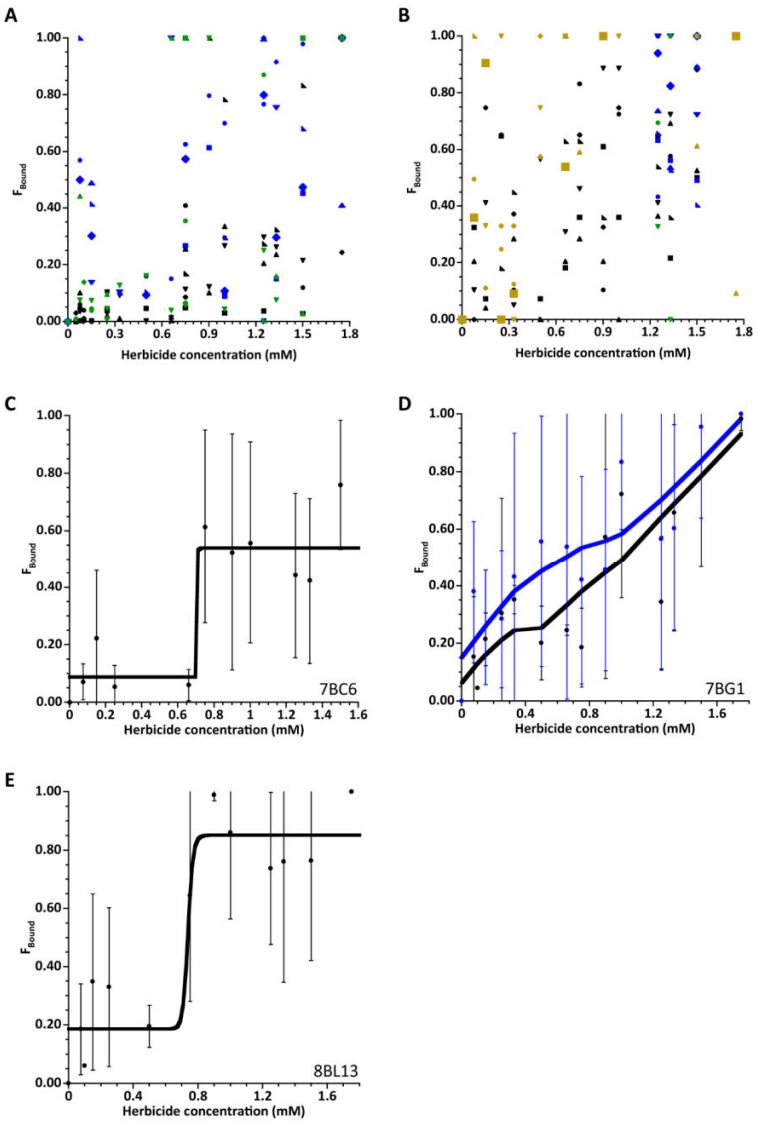

Figure S1. Herbicide concentration-dependent binding studies for control oligos and atrazine-specific aptamers. Oligos were labeled with TAMRA for atrazine and simazine studies and fluorescein for alachlor and metolachlor studies. Oligos were annealed to the immobilized capture oligo prior to introduction of the appropriate herbicide. The starting pool (**A**) and an unstructured oligo (**B**) served as controls. Fluorescence observed in the supernatant represents the aptamer released upon binding (F_{bound}) to atrazine (black), simazine (blue), alachlor (green), and metolachlor (gold). Aptamers 7BC6 (**C**), 7BG1 (**D**), and 8BL13 (**E**) were labeled with TAMRA and annealed to immobilized capture oligo prior to introduction of the appropriate herbicide. Fluorescence observed in the supernatant represents the aptamer released upon binding to the target. Aptamers 7BC6 and 8BL13 showed distinct binding trends with atrazine, while 7BG1 was released with increasing concentrations of atrazine (black) and simazine (blue).

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Table S1. Sequences of DNA aptamers used for testing

Selection pool	GAACTAGATCGCAGC-N_{20}-CGGCAGTGACTCTTGAAC-N_{20}-GGATCGAGGTAATCC
Unstructured	CAACAACAACAACAACAACACGGCAGTGACTCTTGAACCAACAACAACAACAACAACA
7BC1	CCCTGGACGCTAACTGTTTACGGCC**G**GTGACTCTTGAACACTTAACCATATACCCGGGG[a]
7BC2	CCACCCAGCCTACGCAGACACGGCAGTGACTCTTGAACAAAAGAAGTTCTCCTGCATG
7BC3	CACCGTAACGTCAACCGGAACGGCAGTGACTCTTGAACAGTCAACAAGCGTCCGGGTG
7BC6	CCACACCGATGCGGATATGACGGCAGTGACTCTTGAACACTATGTCCTGGTTGCTTGG
7BC7	CACGGAAGGACGGGGTTGGGCGGCAG**C**GACTCTTAACAATCTGGTATCTGTGACTCC[b]
7BC8a	CACCCGCGGTATTGATGCCACGGCAGTGAC**A**CTTGAACACCGTGGGCCTGTGCGTTGG[b]
7BC8b	CCCACAGTCGAGAAGCAGTAAGACGACATGGGAACAAACATGGGGCCAAGAGATCTAGGGCACGCTGTGT[c]
7BC9a	CACACCGGAAGGGAAAAGTTCGGCAGTGA**T**TCTTGAACCAAGTCCCGGTCGCTCGTCG[b]
7BC10	CCCCAGACTAATCCCTACGACGGCAGTGACTCTTGAACGCTCCGCTACAGCTCACGTG
7BC13	TGGAACCCAGCAAGCCATGACGG**T**AGTGACTCTTGAACTTGTCATGCTTTCGTCCTGG[b]
7CB14	ACGACCAATAATGCGGAGCTCGGCAGTGACTCTTGAACCGTTCATATTCGCTCCAGTG
7BE2	CACGGGGGAGCACGATCACAGATTGTCATGGCCTCTATAGTTGGTCTGTCGCCGTGTG[d]
7BE3	CCACCGCACGTCAAACTGCCCG**A**CAGTGACTCTTGAACTTACCGGCATTGGATAGGGG[b]
7BE4	CCCACAACCATGGATTCTAGCGGCAGTGACTCTTGAACTTGGACATATCGAGCGGGGG
7BE5	CCACACCACGACCTTCCCCCCGGCAGTGACTCTTGAACCCGTCGTAAACTCCTACGTG
7BE6	CACACCCAACCCGTGAGCCCCGGCAGTGACTCTTGAACCTCATGAGTTGGCTCCGGTG
7BE7	CGCAGCAAGGAGACCCCAGACGGCAGTGACTCTTGAACAGGCTAACACGGTATTTATG
7BE9	CATAGCCACGCATACTGTTTTCCTAACATGGCTCCTTGAGGTGAACCGTCGCTGGGTG[c]
7BE11	CCCAAGTCCAAGAGTAAGGGCGGCAGTGATCTTAACAATCGTGCTCAGGTCTGGTG[d]
7BE12	CCCCCATAGCTCTCACCCATGCCGGCAGTGACTCTTGAACAAACATCGTTACCGCGCCGG
7BE13	CACACAATCCGGAATCAGAGCGGCAGTGA**T**TCTTGAACTACTTATAGCTTGTGTTGTG[b]
7BG1	CACGGGGGAGCACGGTCACAGATTGTACCCGGCCTCTATAGATGGTCTGTCGCCGTGTG[c]
7BG2	CACGGGGGAGCACGATCACAGATTGTCATGGCCTCTATAGTTGGTCTGTCGACGTGTG[d]
7BG5	ATAGTACGTGAGCGACGTGTCGGCAGTGAC**A**CTTGAACCGGATGATGAAGTGCGCTGG[b]
7BG7	CCGTATGGACCCAAAACTCACGGCAGTG**G**CTCTTGAACTGATCCAATTTCCCCAGGTG[b]
7BG10	CCCAGCCCCCTCGACAGCACC**T**GCAGTGACTCTTGAACCGAATAACCTACGGCATCTG[b]
7BG11	CCACTCGTCGATCCTCATCCCGGCAGTGAC**C**CTTGAACTCCCCCCAATGGCGTAGTGC[b]
7BJ1	CACACGGTGGCACGAGATATCGGCAGTGACTCTTGAACGGTTGTGTGGTGTATGCAGT
7BJ9	CCCAAGGATACCAAAGCCACGGCAGTGACTCTTGAACTTAAATGTAACGCGCTTTGT
7BJ11	AACGCATATTGTGCTGTTCGGCAGTGATCTTGAACTCATACCCTGCTCTGTGTG
7BJ15	CCACGGCGGGACCCGCTAGACGGCAGGGGACTCTTGAACTATCCGTCTGACGCGTGTGGGG[d]
8BL3	CCTTGCGCCCTGAATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCG[c]
8BL13	CCCAGCGTACCGAAAGGGCTCGGCAGTGACTCTTGAACCGTCCCTTGTTTGCTGATGG
8BN10	CACGGGGGAGCACGATCACAGATTGTCATGGCCTCTATAGTTGGTCTGTCGCCGTGTG[d]
8BN11	CACATCGCCTGAGTGCCATTCGGCAGTGACTCTTGAACGCCCGGTCGTCATTGTGCTG
8BN13	CCCACCATCACATCTCTTCGCGGCAGTGACTCTTGAACGCTTAACGAAACCTCTAATG
8BN15	CCACAGCATCACCGTCTATCCGGCAGTGACACTTGAACTTGCCCTTTTCCATCTTGTG
8BN23	ATATCGCCTGAGTGCCATTCGGCAGTGACTCTTGAACGCCCGGTCGTCATTGTGCTG
8BN24	CACACCACTCGAATCATCAACGGCAGTGACTCTTGAACTATCTCTGTCTCGCCACGTG
8BN25	CACATCGGGCCCCCACACAACGGCAGTGACTCTTGAACAGTTTACTGGTCCTGCTGTG
8BN27	CACCCCCCCACCTAGCATCTC**A**GCAGTGACTCTTGAACCAGCACCTCCAGGTCATGTG[b]
8BN28	CACAGCGGGTCGATGGAAGTCGGCAGTGACTCTTGAACACGCCCCAGTCATAGGTGTG
8BN29	CACATCATCTGAGTGCCATTCGGCAGTGACTCTTGAACGCCGGGTCTTGATTGTGCTG

[a]Sequences of the N_{20} regions and capture region (purple) are shown. Tested aptamers include the two primer binding regions flanking the N_{20} regions, (blue & red) separated by the capture region.
[b]Single point mutations are indicated in bold and underlined within the capture region.
[c]No identifiable capture region present.
[d]Capture regions with multiple mutations are shown in green.

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Table S2. Percent elution for selected aptamers tested with various pesticides.

Aptamer	Atrazine	Simazine	Alachlor	Aptamer	Alachlor	Metolachlor	Atrazine
7BC1	22±1	15±2	40±13	7BE2	31±11	11±1	23±7
7BC2	31±2	7±0.3	33±3	7BE3	28±24	40±3	50±7
7BC3	25±2	8±3	23±19	7BE4	29±21	32±9	44±24
7BC6	**48±1**	**20±3**	**15±2**	7BE5	23±12	26±11	30±16
7BC7	19±6	19±3	33±3	7BE6	22±2	63±15	33±16
7BC8a	18±7	18±3	30±8	7BE7	33±14	26±10	35±5
7BC8b	19±8	19±4	50±0	7BE9	34±15	39±3	38±14
7BC9a	17±10	33±0	13±2	7BE11	30±19	27±3	34±7
7BC10	16±5	25±3	22±11	**7BE12**	**28±15**	**33±10**	**50±12**
7BC13	28±16	3±2	35±14	7BE13	52±20	24±8	52±6
7BC14	16±12	12±5	35±8	7BJ1	35±11	26±14	23±8
7BG1	**40±16**	**61±2**	**23±18**	7BJ9	45±16	40±31	49±6
7BG2	42±10	30±6	54±0.3	7BJ11	24±17	24±4	32±13
7BG5	26±11	49±3	53±5	**7BJ15**	**49±18**	**44±1**	**27±10**
7BG7	35±16	23±4	50±27	8BN10	43±12	29±15	36±7
7BG10	30±10	16±6	37±2	8BN11	25±3	NA[a]	32±2
7BG11	45±11	29±9	44±4	8BN13	38±2	30±11	14±2
8BL3	17±8	31±11	22±3	**8BN15**	**44±4**	**40±3**	**18±4**
8BL13	**35±15**	**17±17**	**19±19**	**8BN23**	**56±6**	**39±1**	**29±16**
				8BN24	48±14	52±15	55±16
				8BN25	47±9	38±22	28±3
				8BN27	27±7	21±4	23±2
				8BN28	37±8	14±7	42±2
				8BN29	**49±18**	**37±23**	**17±17**

[a]No measurable elution.

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