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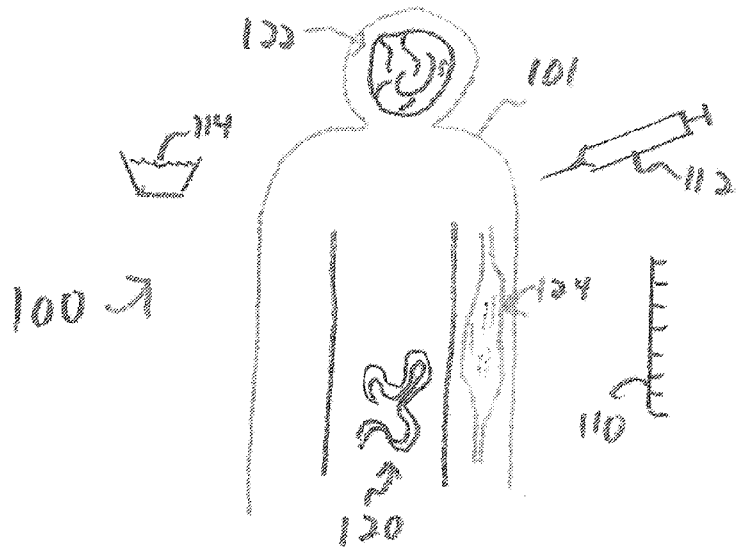

Fig. 1

(57) **Abstract:** An aptamer having an affinity for toxic metals such as lead is introduced by a biocompatible delivery mechanism such as a DNA or RNA strand to which the aptamer is attached. The delivery mechanism delivers the aptamer, either as a direct nucleic acid sequence or expressed in a cell as a probiotic. When delivered as a prophylactic to the gastrointestinal tract (orally) as an aptamer or expressed within a probiotic cell, this would prevent absorption of metals and would thus reduce or eliminate the need for chelation therapy and thereby reduce disease burden. When used therapeutically, it could be ingested, or injected intravenously. Once bound, the toxic metals are expelled through normal gastrointestinal or urinary processes.

[Continued on next page]

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HEAVY METAL TOXICITY REMEDIATION

BACKGROUND

Lead (Pb(II)) poisoning remains a global public health problem, causing neurodevelopmental anomalies in children and increased risk of cardiovascular, renal, and neurological disease in adults. The current standard of treatment for lead toxicity is chelation therapy with oral medication, or EDTA (Ethylenediaminetetraacetic acid disodium salt dihydrate) chelation by intravenous administration. However, these therapies are typically only offered in the case of extremely high lead levels (blood lead levels above 45 ug/dL for children and 70 ug/dL for adults), even though much lower levels (3.5 ug/dL for children and 5 ug/dL for adults) are associated with negative health consequences. The neurotoxic effects of lead are permanent. Lead exposures are cumulative, and deposits into bones and can take years to be fully eliminated from the body.

SUMMARY

An aptamer having an affinity for toxic metals such as lead is introduced by a biocompatible delivery mechanism such as a DNA or RNA strand to which the aptamer is attached. The delivery mechanism delivers the aptamer, either as a direct nucleic acid sequence or expressed in a cell as a probiotic. When delivered as a prophylactic to the gastrointestinal tract (orally) as an aptamer or expressed within a probiotic cell, the aptamer effectively prevents absorption of metals and would thus reduce or eliminate the need for chelation therapy and thereby reduce disease burden. When used therapeutically, it could be ingested, or injected intravenously. Once bound, the toxic metals are expelled through normal gastrointestinal or urinary processes.

Configurations herein are based, in part, on the observation that heavy metals such as lead are associated with negative health symptoms, and tend to cause cumulative negative effects that are problematic to reverse. Unfortunately, conventional approaches to heavy metal toxicity suffer from the shortcoming that treatment is not pursued until already harmful levels have been absorbed, and because it is problematic to remove the absorbed toxins; rather, mere mitigation of additional intake is pursued. Accordingly, configurations herein substantially overcome the shortcomings of conventional approaches by providing an aptamer configured to bind with heavy metals such as lead, and deliver the aptamer to a patient physiology by DNA or RNA mediums, where the aptamer fragment is available to bind with the heavy metal, following which it is expelled as waste.

Many lead exposures are via ingestion from environmental sources: water, food, or incidental ingestion of contaminated dust or paint chips. When the water or working conditions are to blame, it may be difficult or impossible to eliminate lead from the environment completely. The neurotoxic effects of lead are permanent, leading to lifelong cognitive deficits in these children and creating a disease burden that is borne disproportionately by racially diverse and low-income communities. Therefore, there is not only an urgent need but an environmental justice obligation to develop accessible and cost-effective methods to protect people from lead.

Additional uses can be derived according to configurations herein by designing a binding aptamer targeting a toxicity source such as presented by a toxic metal, and implementing the biocompatible delivery mechanism for introducing the binding aptamer.

In further detail, configurations herein, a method of prophylactic and therapeutic treatment of metal toxicity such as lead includes determining a binding aptamer having an affinity for a toxic metal, and generating a nucleic acid strand including the binding aptamer. The generated nucleic acid strand is delivered into a therapeutic region for binding and transport of the toxic metal, and subsequent elimination thought normal physiologic processes.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, features and advantages of the invention

will be apparent from the following description of particular embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

Fig. 1 is a context diagram of a treatment and remediation environment suitable for use with configurations herein;

Fig. 2 is a depiction of aptamer binding to a toxic metal for fluorescence based detection in the environment of Fig. 1;

Fig. 3 is a graph of mitigation of a toxic metal presence according to configurations herein;

Fig. 4 is a graph showing the efficacy of the Pb7S lead-binding DNA aptamer used as a prophylactic for reproductive toxicity of lead;

Fig. 5 is a graph showing the efficacy of the Pb7S lead-binding RNA aptamer used as a prophylactic for reproductive toxicity of lead;

Fig 6 is a graph showing the efficacy of the Pb7S lead-binding DNA aptamer used as a prophylactic for neurotoxicity;

Fig. 7 is a process flow of delivery of an aptamer and introduction into a treatment regimen for binding with a toxic metal; and

Fig. 8 shows an extension of the binding aptamer used as a prophylactic for hexavalent chromium (Cr(VI)) toxicity.

DETAILED DESCRIPTION

Heavy metal toxicity is associated with significantly increased risks of cancer and cardiovascular disease, affecting many millions of Americans, and hundreds of millions globally. Most adult humans have significant bioaccumulation of toxic metals (lead, cadmium, arsenic, and others). Despite the fact that we have ample scientific evidence to support the role of toxic metals in increased human disease risk, typically no medical intervention is taken for metal toxicity unless the exposure is so high as to be causing acute disease. The current standard of care for removing heavy metals from the human body is chelation therapy with EDTA disodium. This medication is delivered intravenously, and therefore requires

medical supplies and professionals to deliver the treatment, typically in a doctor's office.

Lead causes neurodevelopmental anomalies in children and increased risk of cardiovascular disease (CVD), renal damage, and neurological disease in adults. Lead exposures are cumulative, and the neurologic damage caused is permanent. The molecular mechanism of lead toxicity is related to its ability to replace calcium in biological processes. Lead enters cells through calcium channels and can interfere with calcium ion flow, which is a central mechanism of its neurotoxic effects. Lead integrates into hydroxyapatite and can be stored for many years in bone, leaching back into the body even after exposures are eliminated and thus taking years or decades to be fully cleared from the body.

Aptamers are small DNA- or RNA-based oligonucleotides which are typically produced by the systematic evolution of ligands by exponential enrichment (SELEX) technology. Aptamers are short stretches of nucleic acids (<100 nucleotide single-stranded DNA or RNA molecules) that bind specifically to a target molecule or ion. Under certain conditions, aptamers can fold into three-dimensional structures. Structural motifs within aptamers provide specific binding sites for small molecules or macromolecular compounds of several types, including cells, cell surface proteins, bacteria, and viruses; moreover, they interact with targets with high affinity and selectivity. Aptamers are sometimes referred to as chemical antibodies, but they have huge advantages over them, like increased stability, less expensive and less time-consuming production, ease of chemical modification, lower immunogenicity, and higher target range.

Certain aptamers, known as Pb7 and Pb14, have been shown to have low micromolar to high nanomolar affinity (1.60 ± 0.16 μM and 0.76 ± 0.18 μM, respectively) for lead ions and reported minimal cross-reactivity to other ions. Structural analyses suggest that lead can associate with nucleic acids through so-called G-quadruplex (G4) structures. Most lead-binding aptamers have been developed for the purpose of lead detection in environmental and biological samples.

The affinity of aptamers to bind to lead in vitro has previously been shown for purposes such as water supplies. Conventional approaches do not address the

prophylactic use of aptamers in an organism to prevent metal toxicity. Development of aptamer-based approaches to prevent gastrointestinal absorption of lead represents a significant improvement over reactive treatments such as chelation therapy, which cannot reverse lead- induced tissue damage. The application of nucleic acid aptamers to the prevention of heavy metal toxicity is innovative and has not previously been demonstrated.

Configurations herein demonstrate that an aptamer having an affinity for a particular metal toxin provides selective binding with the targeted metal toxin for subsequent elimination. The aptamer need only be delivered as a strand or fragment from a probiotic or other suitable biocompatible delivery mechanism. Configurations herein demonstrate effects on nematode *Caenorhabditis elegans* (*C. elegans*) which is a ~1 mm transparent soil organism that has been commonly used as a laboratory model organism. The *C. elegans* model, demonstrates that that lead-chelating DNA and RNA aptamers applied in the presence of lead protect the animals from reproductive and behavioral toxicity. Both DNA and RNA versions of the aptamers are effective, and the protective effect is specific to lead and to the aptamer sequence. Similar approaches show that aptamers protect cultured cells from lead toxicity, and protect osteoblastic function. Such aptamer-based chelation can be further developed as a prophylactic or therapeutic strategy for human exposures to toxic metals by selecting an aptamer having an affinity for a specific, targeted toxic metal.

Fig. 1 is a context diagram of a treatment and remediation environment 100 suitable for use with configurations herein. In a patient 101 afflicted with toxic metal poisoning, the disclosed method of prophylactic and therapeutic treatment of metal toxicity includes determining a binding aptamer 110 having an affinity for a toxic metal, and generating a nucleic acid strand including the binding aptamer, such as a DNA or RNA for use as a biocompatible transport mechanism. An injected 112 or orally ingested 114 form of the generated nucleic acid strand is delivered into a therapeutic region for binding and transport of the toxic metal. This permits binding the binding aptamer to engage and bind with the toxic metal to form a bound aptamer, and expulsion of the bound aptamer via the urinary tract or gastrointestinal tract. For prophylactic treatment, the binding aptamer in the gastrointestinal region

120 binds with the toxic metal for excretion prior to any bodily absorption. For therapeutic treatment, presence of the nucleic acid strand with the binding aptamer 110 in the brain/central nervous system (CNS) 122 or musculature 124 will attract and bind to the toxic metal in tissue, and successive courses of treatment will tend to diffuse concentrations of the toxic metal out via the bloodstream, for example, and ultimately for extraction via the kidneys and urine.

One of the advantages of aptamers is the binding selectivity. Aptamers can be engineered to attract and bind specific targeted molecules. In the disclosed approach, lead remediation is a particularly beneficial approach, because lead tends to mimic calcium in human physiology, which facilitates migration into bones, in addition to other harmful anomalies. A binding aptamer is engineered that has a greater affinity for lead than for calcium, as it is important to not only expel the lead, but also to avoid collateral effects with normal biochemical processes. Potential binding aptamers may be selected to target a number of toxic metals, including but not limited to Pb, Cd, Co, Cr, Hg, Mn, Se, Fe, Ba, Be, Cs, Cu, Pt, Sb, Sn, Tl, V, Ni, U and W.

Fig. 2 is a depiction of aptamer binding to a toxic metal in the environment of Fig. 1. Referring to Figs. 1 and 2, lead-binding single stranded (ss)DNA aptamers Pb7 and Pb14 (Table I) have been shown to have beneficial lead attraction potential. These aptamers were originally selected for use in a fluorescence-based detection assay for lead used for lead contamination in drinking water. The affinity for lead, or other toxic metals, can be leveraged by introducing a binding aptamer into a patient using a suitable biocompatible delivery mechanism, typically including a DNA or RNA based form in a controlled therapeutic or prophylactic approach. In the example of Fig. 2, the affinity of the binding aptamer is shown where the aptamers were 5' end labeled with a fluor (fluorescein amidite, FAM) 201, and annealed to a shorter antisense strand with a 3' quencher (dabcyl, DAB) 203, which quenched the fluorescent output. Upon aptamer binding to lead ions 205, the quench strand 207 was released, resulting in a fluorescent signal. In Fig. 2, 5' FAM labeled aptamers are hybridized with short 3' DAB labeled quench strands to form a partial double helix at the 5' end of the aptamer. The proximity of the DAB to FAM quenches fluorescent signal. Upon addition of Pb(II) 205, the FAM-aptamer

dissociates from the quench strand and forms a G quadruplex 210 structure around the lead ion, releasing the DAB-quench strand and resulting in detectable fluorescence.

Aptamer	Length (nt)	Sequence
Pb7	76	GGAGGCTCTCGGGACGACGGCAGGGCTGTCGTACGGTTTGTCGAAGGTGTCG TCCCGATGCTGCAATCGTAAGAAT
Pb7S	48	GGGACGACGGCAGGGCTGTCGTACGGTTTGTCGAAGGTGTCGTCCCGA
Pb7S antisense	48	TCGGGACGACACCTTCGACAAACCGTACGACAGCCCTGCCGTCGTCCC
Pb7S scrambled	48	GCGGGCGATCTGCGGACGTTCTGAGCCTGACTGAGTGGGGACGCTGTA

TABLE I

To confirm lead binding to the Pb7S aptamer, we reproduced the fluorescence-based lead binding assay, testing combinations of two flours (FAM and Yakima Yellow) and three quenchers (DAB, Black Hole Quencher 1 (BHQ1), and Iowa Black (IAB)) . Using the Yakima Yellow fluor, we found a statistically significant difference in fluorescence from the no lead control at 20 μM lead, indicating an interaction of lead with the aptamer. The fluorescence detection system was highly sensitive to pH, with lower (pH 5.5) and higher (pH 8.4) values resulting in a loss of dynamic range, which was suspected as a caveat of using fluorescent detection, rather than a pH-dependent association of lead with the aptamer.

The conventional aptamer use incorporating fluorescence indicators covers detection only. Such a fluorescence labeling, quench strand, and the like are not used prophylactically or therapeutically. For human intervention, effective delivery vehicles as well as confirmation of no or merely acceptable side effects must also be established.

In a human or mammalian context, the delivery mechanism would deliver the aptamer in the form of DNA or RNA strand. The delivery mechanism to introduce the aptamer into the human physiology may be in the form of a capsule, therapeutic virus, probiotic bacteria, lipid nanoparticle, or other nanomaterials. The aptamer may be inside of the biocompatible delivery mechanism, or may be covalently or non-covalently attached to it. The aptamer may be released from the delivery mechanism or may remain within or bound to the delivery mechanism.

Fig. 3 is a graph of mitigation of a toxic metal presence according to configurations herein. Structural modeling predicts the formation of a G-quadruplex (G4) structure in the Pb7S aptamer. Lead ions are known to assemble into G4s with high affinity, creating unique G4 signatures by circular dichroism (CD) spectroscopy. To confirm the specific interaction of the Pb7S aptamer with lead ions in a manner independent from fluorescence detection, we applied CD to measure the lead-dependent assembly of the G4, shown in Fig. 3. The unbound Pb7S aptamer 301 and scrambled control 302 displayed a strong CD maximum in a single peak at 280nm. The addition of lead ions to the Pb7S aptamer 303, but not the scrambled control, resulted in the concentration-dependent appearance of a broad peak with a maximum at 314 nm, reflective of the organization of a G4 structure. The formation of the peak at 314 nm was identical when the pH of the solution used was 5.5 or 8.4, suggesting that the interaction is not particularly sensitive to pH in this range. As lead mimics calcium within biological systems, we investigated the binding of the Pb7S aptamer to calcium, and found no evidence of G4 formation in response to calcium. Further, the presence of calcium did not alter the formation of the G4 structure when lead was added subsequently. From the above CD experiments, it can be concluded that lead ions bind with high specificity to the Pb7S aptamer through the formation of a G4 structure.

Referring again to the *C. elegans* experiments above, lead has previously been shown to result in reproductive toxicity in *C. elegans*, causing a dose-dependent decrease in brood size. These prior studies were conducted with animals exposed to metals by continuous growth in liquid cultures in multi-well plates. To better mimic dietary exposure to metals, we chose to expose our animals to metals by feeding. We first confirmed the dose-dependent decrease in brood size using our experimental feeding method. L3 stage animals were plated to NGM agar seeded with their food source OP50 *E. coli* mixed with lead acetate at concentrations from 0 – 25 mM. We found by this method that 15 mM lead exposure in the OP50 lawn was sufficient to cause an approximately 50% decrease in brood size.

Fig. 4 is a graph 400 showing the efficacy of the Pb7S lead-binding DNA aptamer used as a prophylactic for reproductive toxicity of lead. To determine whether chelation of lead ions with aptamers could reduce reproductive toxicity, we

employed three strategies to expose the animals to the aptamer: feeding, soaking, and drop casting. The feeding strategy mixed the aptamers at the designated concentration into the OP50, with or without lead, then animals were plated to this mixture and offspring were counted. The soaking strategy exposed animals to aptamers in an aqueous solution for 2.5 hours, then the animals were moved to NGM (Nematode Growth Medium) plates seeded with OP50 with or without lead. For the drop casting method, animals were plated to NGM plates containing OP50 with 402, 403, 404 or without 401 lead, then 10μL of aptamer at the indicated concentration was dropped onto the animal. We observe by all methods (the drop cast method results are shown in Fig. 4) the Pb7S DNA aptamer result in protection of animals from lead-induced reproductive toxicity 403, whereas antisense (reverse complement strand DNA) controls 404 have no effect on brood size reduction caused by lead.

To thoroughly examine the protective effect of the Pb7S aptamer, the aptamer was tested at a range of both aptamer and lead concentrations. The minimum effective concentration of aptamer required to achieve full protection from exposure at 15mM lead acetate was 2.5 μM. At 2.5 μM treatment, significant protection of animals was observed up to 100 mM lead acetate. Therefore, the results demonstrate the specific, dose-dependent protection of animals from ingested lead toxicity by exposure to lead-binding ssDNA aptamers.

Having determined an binding aptamer having an affinity for lead ions (human absorbed lead is typically Pb(II), or a Pb^{2+} ion form), and that favorable protection and extraction of lead was observed in laboratory trials, a delivery mechanism compatible with human physiology is called for. Modified RNAs (siRNAs and mRNAs) have been approved in the U.S. for therapeutic and prophylactic uses, and are a promising treatment modality.

Fig. 5 is a graph 500 showing the efficacy of the Pb7S lead-binding RNA aptamer used as a prophylactic for reproductive toxicity of lead. To determine whether an RNA version of the Pb7S aptamer could also efficiently protect *C. elegans* from reproductive toxicity, we repeated our brood size assays using RNA versions of Pb7S and scrambled controls using the drop casting method. RNA aptamers 503 result in protection of animals from lead-induced reproductive

toxicity, similar to lead negative samples 501, whereas scrambled 505 and antisense 504 controls have no effect on brood size. To examine the protective range of the RNA Pb7S aptamer, we tested the aptamer at a range of both aptamer and lead 502 concentrations. The minimum effective concentration of the RNA aptamer required to achieve full protection from exposure at 15mM lead acetate was 2.5 μM. At 2.5 μM treatment, significant protection of animals was observed up to 100 mM lead acetate. These ranges were identical to those revealed in our ssDNA Pb7S aptamer testing. We conclude that ssRNA PB7S aptamers are equally as effective as ssDNA aptamers in protecting *C. elegans* from reproductive toxicity. Both DNA and RNA configurations can therefore be used for combining the nucleic acid strand with a biocompatible delivery mechanism for introduction into the subject patient.

Fig 6. is a graph 600 showing the efficacy of the Pb7S lead-binding DNA aptamer used as a prophylactic for neurotoxicity. Early lead exposure in children is well established to result in developmental neurotoxicity. We therefore sought to employ a model of developmental neurotoxicity in the form of a behavioral assay in our *C. elegans* model. *C. elegans* are known to move away from aversive cues, a pattern of behavior known as avoidance. To determine whether lead exposure negatively impacted *C. elegans* avoidance behavior during larval development, we exposed L1 stage worms to lead, then allowed them to develop to the L3/L4 stage in the presence of lead, and tested their avoidance of a noxious chemical cue. As shown in Fig. 6, lead exposure during larval development resulted in a dampened avoidance response to all noxious cues 602 as opposed to a normal avoidance response 601. Exposure to the Pb7S DNA aptamer in the absence of lead 603 had no effect on the normal avoidance behavior, suggesting the aptamer itself is not neurotoxic. When the animals were exposed to the Pb7S DNA aptamer in addition to the lead 604, there was a restoration of the normal avoidance behavior. The results suggest that the aptamer protects the animals from lead-induced neurotoxicity during development. The leftmost bar in each sample is a solvent control measure.

To determine whether ssDNA Pb7S aptamer could protect mammalian cells from lead toxicity, we used cell proliferation assays to measure the effect of lead on cultured cell growth. To utilize Pb7S in a human patient setting, a biocompatible vehicle needs to be generated for transporting the nucleic acid sequence including

introducing the binding aptamer into a subject patient for remediation. Several approaches may be employed. An RNA therapeutic can be formed including the nucleic acid strand. Also, a probiotic approach can form a probiotic including the nucleic acid strand by appending the nucleic acid strain to a DNA strand; and replicating the DNA strand including the binding aptamer, Pb7S in the disclosed example. Then the DNA would be transcribed into multiple copies of an RNA aptamer for targeting the toxic metal. A suitable approach includes adding or editing the DNA of the probiotic, bacteria, or other biocompatible organism to contain the sequence of the aptamer strand.

As indicated above, the current standard of treatment for lead toxicity is chelation therapy with oral medication, or EDTA chelation by intravenous administration. However, these therapies are typically only offered in the case of extremely high lead levels (blood lead levels above 45 µg/dL for children and 70 µg/dL for adults), despite the fact that much lower levels are associated with negative health consequences, as discussed above. The recommended course of action for lower blood lead levels (3.5 – 45 µg/dL) is to continue to monitor the lead levels of the patient and attempt to identify and eliminate the source of contamination. Again, this course of action cannot reverse permanent neurologic damage, nor can it prevent the accumulation of lead in bones. Interventions to protect exposed individuals against low amounts of lead are lacking and are urgently needed.

Fig. 7 is a process flow of the disclosed aptamer and introduction into a treatment regimen; and generating a biocompatible vehicle, such as a DNA or RNA or other suitable structure, to introduce the binding aptamer into a patient physiology. As disclosed above, the biocompatible delivery mechanism may be any suitable therapeutic virus, probiotic bacteria, lipid nanoparticle, or other nanomaterials. The delivery vehicle, while not exclusively RNA or DNA, would contain or deliver the aptamer, which includes the RNA or DNA. Referring to Figs. 1 and 4, the binding aptamer 110 is generated, developed or identified to have an affinity for binding to a target toxin, such as lead. An editing or sequencing application is employed to form a biocompatible delivery vehicle 150, such as an RNA or DNA strand with the binding aptamer 110 included. The biocompatible

delivery mechanism is employed for delivery of the binding aptamer 110 (aptamer). The binding aptamer may be inside of the biocompatible delivery mechanism 150-A, may be covalently or non-covalently attached to it 150-B or genetically expressed within a living delivery system such as a probiotic bacteria 150-C. For any delivery vehicle, the aptamer may be released 160-1 from the delivery vehicle or may remain within 160-2 or bound to 160-3 the delivery mechanism.

In general, the treatment involves a therapeutic compound with a nucleic acid strand including a binding aptamer, such that the binding aptamer has an affinity for a toxic metal, and a biocompatible delivery vehicle including at least one of a DNA or RNA structure, where the structure includes the binding aptamer. Any suitable biocompatible delivery mechanism may be employed. Various derivative or alternative DNA or RNA chemistries, included but not limited to ribose or deoxyribose sugar ring modifications (e.g., locked nucleic acids (LNAs), 2'-O-methyl, 2'-O-methoxyethyl), base substitutions (e.g., pseudouridine), left-handed or "mirror" DNA (L-DNA), backbone modifications (e.g., phosphorothioate (PS), Thiophosphoramidate, Morpholino), and glycosylated nucleic acids may be employed.

Whatever biocompatible delivery mechanism is employed, the binding aptamer may be appended to a strand of the biocompatible delivery vehicle, as an addition to a DNA or RNA strand, or may be in the form of a probiotic including cells 165 having DNA with a strand of the binding aptamer included in the DNA. Other suitable biocompatible delivery mechanisms may be employed for introducing the binding aptamer into a patient physiology, such as formulation into a lipid nanoparticle for injection, or encapsulation into a tablet or capsule for oral delivery (in addition to introduction by a probiotic bacterial or yeast strain).

Returning to Fig. 7, upon introduction into a patient physiology 701, the binding aptamer 110' has an affinity for binding with the target toxin and binds or "wraps" around the target toxin 155, such as by a forming a G-quadraplex from the combination of the now-bound aptamer 110" with the target toxin 155. Other suitable binding approaches may be employed, based on the selectivity and affinity of the binding aptamer to dissociate from a molecule defining the delivery mechanism 150 and form a bond with the target toxin. The bound toxin is then

capable of removal by patient physiology as waste, via the kidneys or GI tract.

In the case of prophylactic measures, it is expected that a GI presence of the binding aptamer can eliminate lead prior to absorption into tissue. Subsequent to absorption, however, intravenous or tissue presence of the aptamer can still draw the target toxin from tissue based on the affinity and normal diffusion in a therapeutic approach. In the case of Pb7S and lead, the selectivity of the binding aptamer is such that beneficial calcium will not be targeted, even though the emulation of calcium by lead is a common result of lead poisoning.

Fig. 8 shows an extension of the binding aptamer used as a prophylactic for hexavalent chromium (Cr(VI)) toxicity. It has been shown that highly toxic Cr(VI) causes a hyper-stimulatory behavioral phenotype in the earthworm species *Eisenia fetida* (PMIDs:15978294, 29621711). Using the same methods described in this proposal, we have confirmed: a) that Cr(VI) causes a hyper-stimulatory aversive behavioral response in *C. elegans* which is consistent with phenotypes observed in earthworms; and b) that Cr(VI)-binding aptamers, but not antisense or scrambled controls, prevent this hyper-stimulatory phenotype and provide protection against Cr(VI)-induced behavioral toxicity. The result demonstrates the protective action of aptamers in a context where the behavioral anomaly is distinct from that caused by Pb(II). This result is significant because it supports our claim that aptamer- based prophylactic strategies could be useful against a range of environmental toxicants that cause variable toxic phenotypes. Our results with lead-binding aptamers are therefore not an anomaly, but the discovery of a novel application for aptamers that is likely to have broad impacts on public health.

Fig. 8 illustrates hyper-stimulatory behavioral phenotype caused by hexavalent chromium exposure is prevented by chromium-binding aptamer. L1-stage worms were plated to N2 plates, with or without 3.5mM Cr(VI) as indicated, and with or without 100 μM chromium aptamer or scramble control. 10mM copper chloride was applied to the animal as a noxious stimulus, and an aversive response was recorded (reverse movement away from the stimulus). Solvent controls (left bar, water only) were included for each trial condition and produced a minimal aversive response. n=6 experimental replicates, 10 worms/plate x 3 plates per condition were tested within each experimental replicate.

While the system and methods defined herein have been particularly shown and described with references to embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A method of prophylactic and therapeutic treatment of metal toxicity, comprising:

determining a binding aptamer having an affinity for a toxic metal;

generating a nucleic acid strand including the binding aptamer; and

delivering the generated nucleic acid strand into a therapeutic region for binding and transport of the toxic metal.

2. The method of claim 1 further comprising:

binding the binding aptamer with the toxic metal to form a bound aptamer; and

expelling the bound aptamer via a urinary tract or gastrointestinal tract.

3. The method of claim 1 further comprising forming a G-quadraplex from the combination of the binding aptamer with lead.

4. The method of claim 1 wherein the binding aptamer has a greater affinity for lead than for calcium.

5. The method of claim 1 further comprising combining the nucleic acid strand with a biocompatible delivery mechanism for introduction into a subject.

6. The method of claim 1 further comprising generating a biocompatible vehicle for transporting the nucleic acid sequence including introducing the binding aptamer into a subject for remediation.

7. The method of claim 5 further comprising forming an RNA therapeutic including the nucleic acid strand.

8. The method of claim 5 further comprising forming a probiotic including the nucleic acid strand by:

manipulating a DNA strand to contain the sequence of the binding aptamer; and

replicating the DNA strand including the binding aptamer.

9. The method of claim 1 wherein the toxic metal is selected from the group consisting of Pb, Cd, Co, Cr, Hg, Mn, Se, Fe, Ba, Be, Cs, Cu, Pt, Sb, Sn, Tl, V, Ni, U and W.

10. The method of claim 1 wherein the binding aptamer is Pb7S.

11. A therapeutic compound, including:

a nucleic acid strand including a binding aptamer, the binding aptamer having an affinity for a toxic metal; and

a biocompatible delivery vehicle having at least one of a DNA or RNA structure including the binding aptamer.

12. The device of claim 11 wherein the binding aptamer is appended to the biocompatible delivery vehicle.

13. The device of claim 11 further comprising a probiotic, the probiotic including cells having DNA with a strand of the binding aptamer included in the DNA.

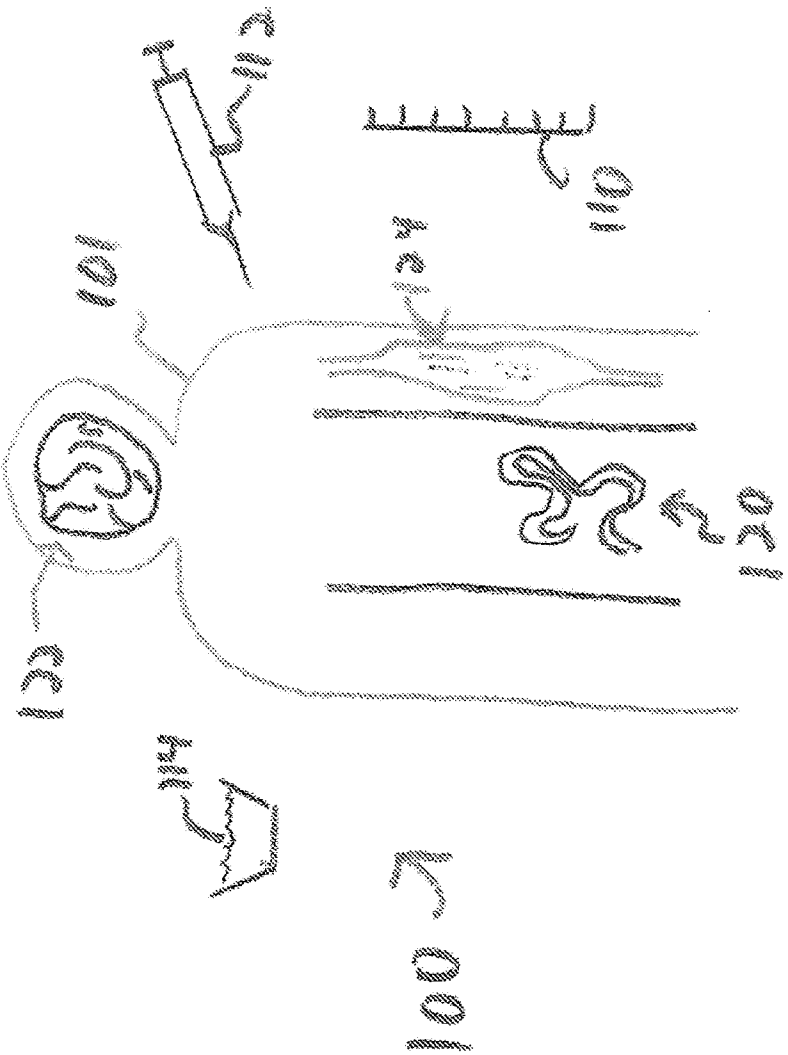


Fig. 1

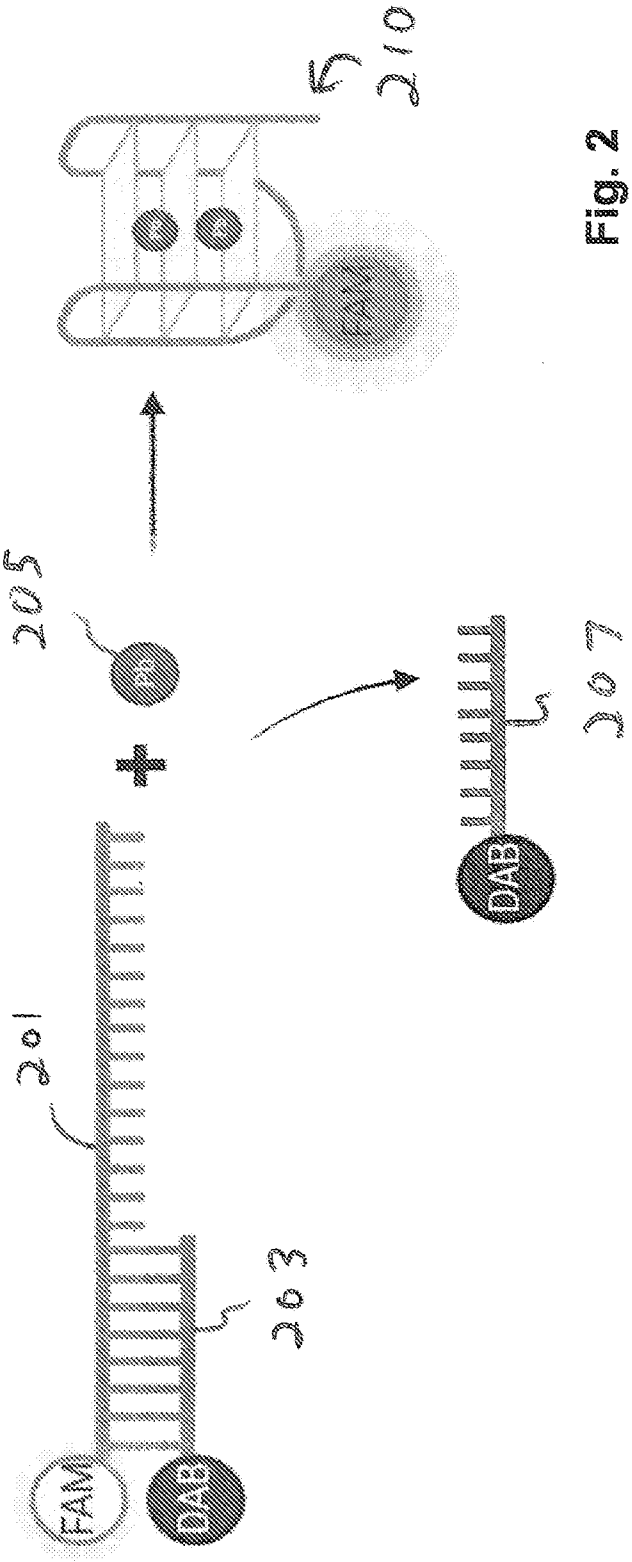


Fig. 2

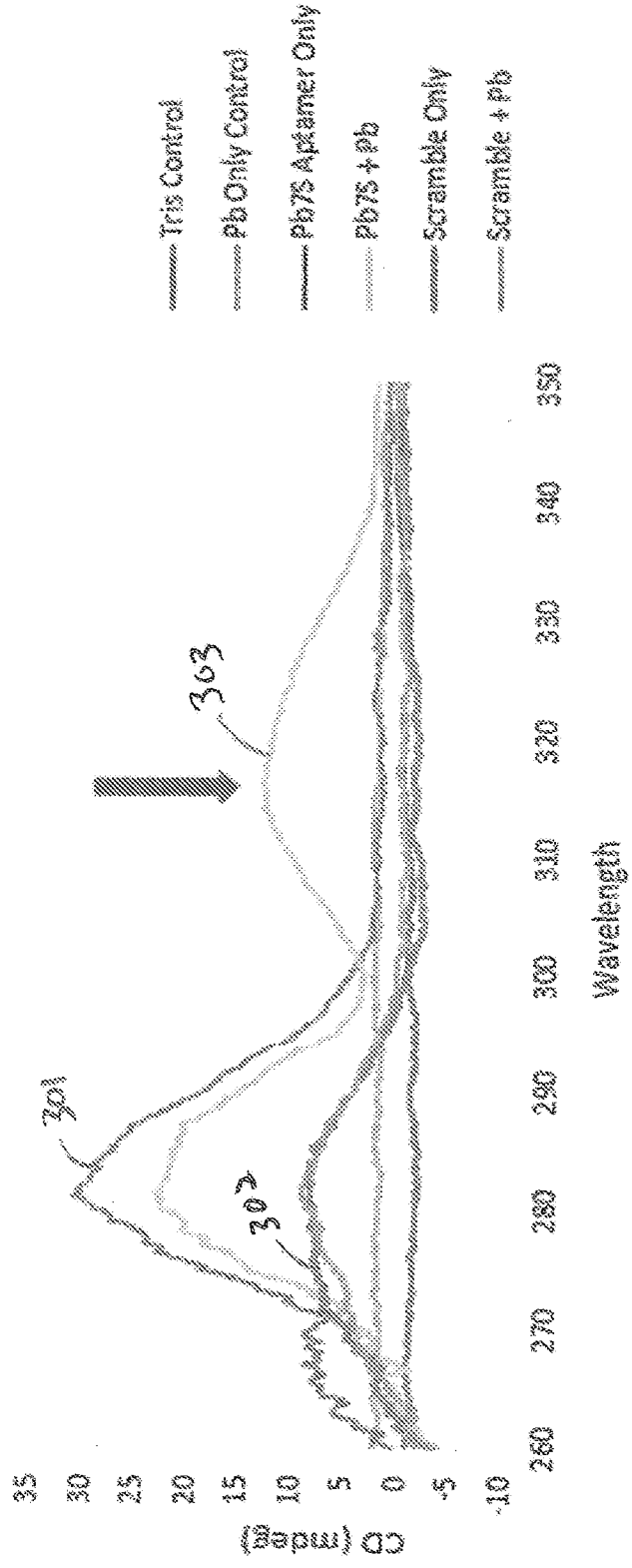
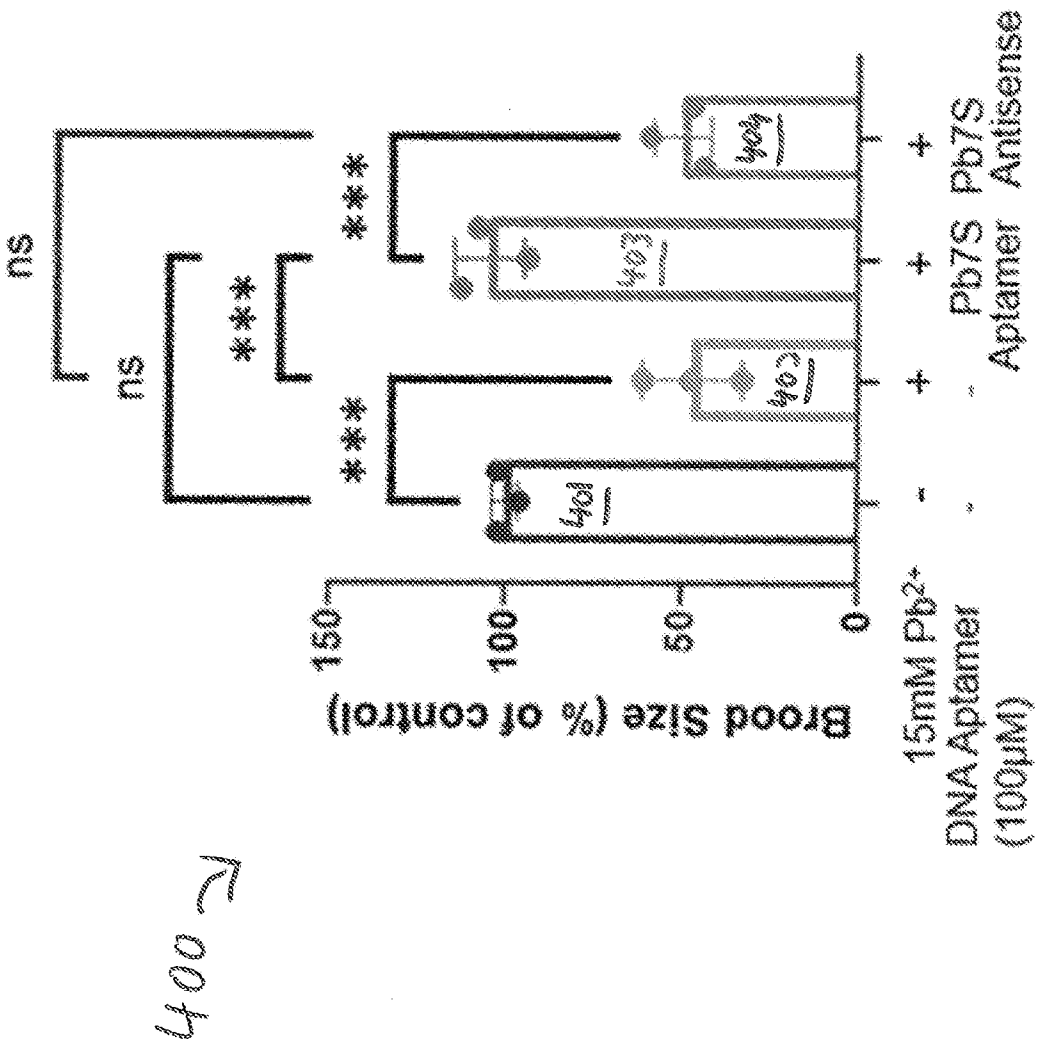

Tris Control
Pb Only Control
Pb7S Aptamer Only
Pb7S + Pb
Scramble Only
Scramble + Pb
301
303
307
CD (mdeg)
35
30
25
20
15
10
5
0
-5
-10
260
270
280
290
300
310
320
330
340
350
Wavelength

Fig. 3


Brood Size (% of control)
150
100
50
0
ns
****
ns
****
****
401
403
405
15mM Pb2+
DNA Aptamer
(100μM)
-
+
+
+
Pb7S
Aptamer
Pb7S
Antisense

400

Fig. 4

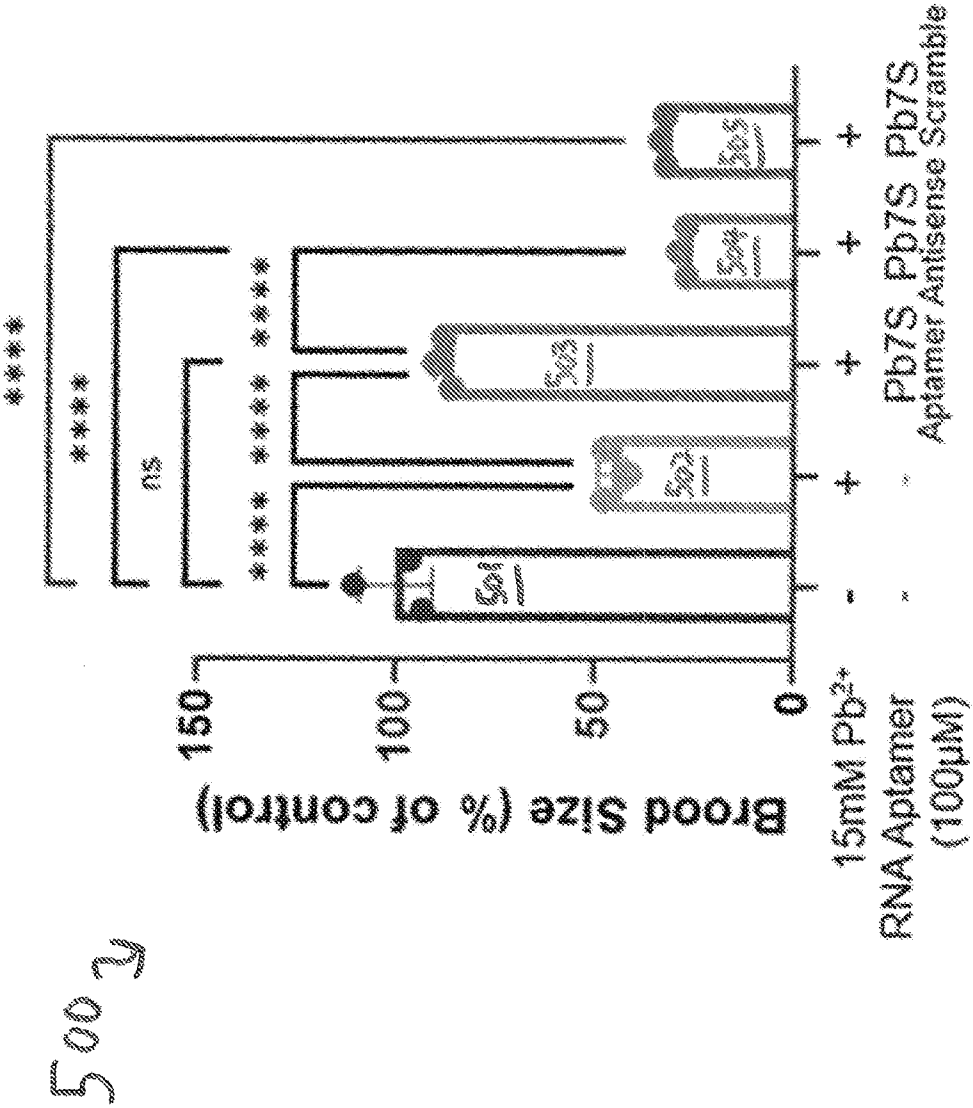

Brood Size (% of control)
150
100
50
0
****
****
ns
****
****
****
15mM Pb2+
RNA Aptamer (100µM)
-
+
+
+
+
Pb7S
Pb7S
Pb7S
Aptamer
Antisense
Scramble

Fig. 5

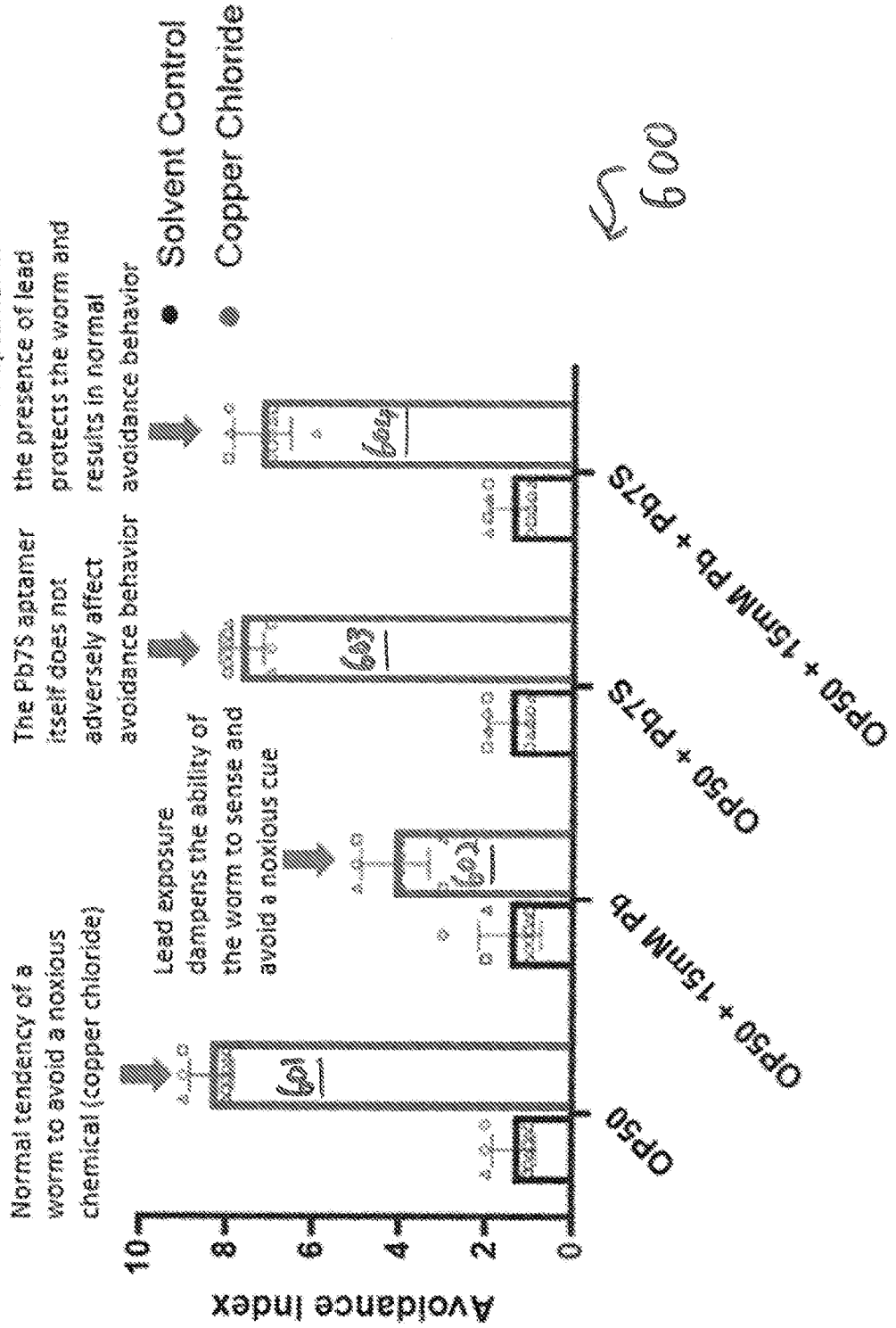

Normal tendency of a worm to avoid a noxious chemical (copper chloride)
Lead exposure dampens the ability of the worm to sense and avoid a noxious cue
The Pb7S aptamer itself does not adversely affect avoidance behavior
The Pb7S aptamer in the presence of lead protects the worm and results in normal avoidance behavior
Solvent Control
Copper Chloride
Avoidance Index
10
8
6
4
2
0
OP50
OP50 + 15mM Pb
OP50 + Pb7S
OP50 + 15mM Pb + Pb7S
600

Fig. 6

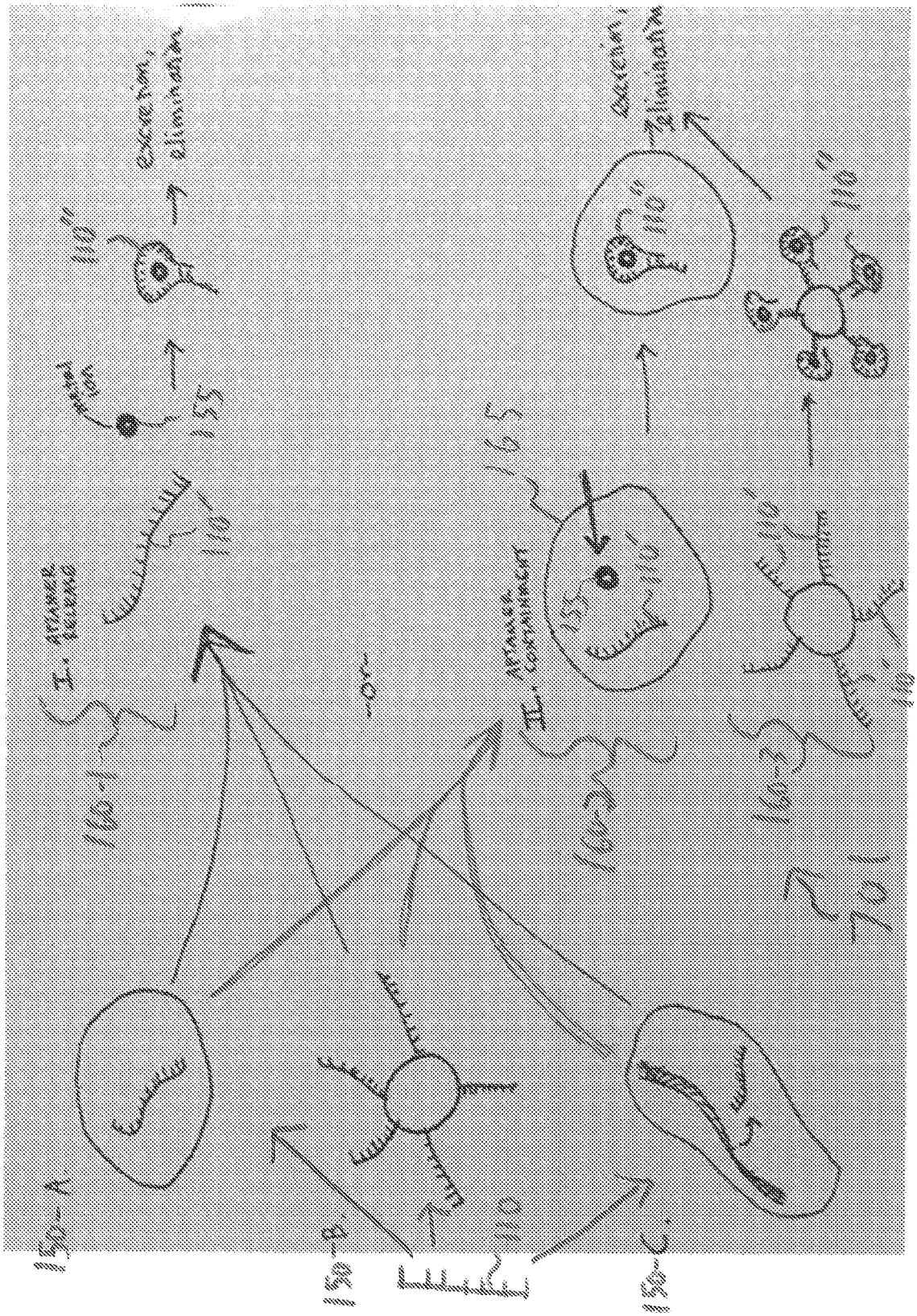


Fig. 7

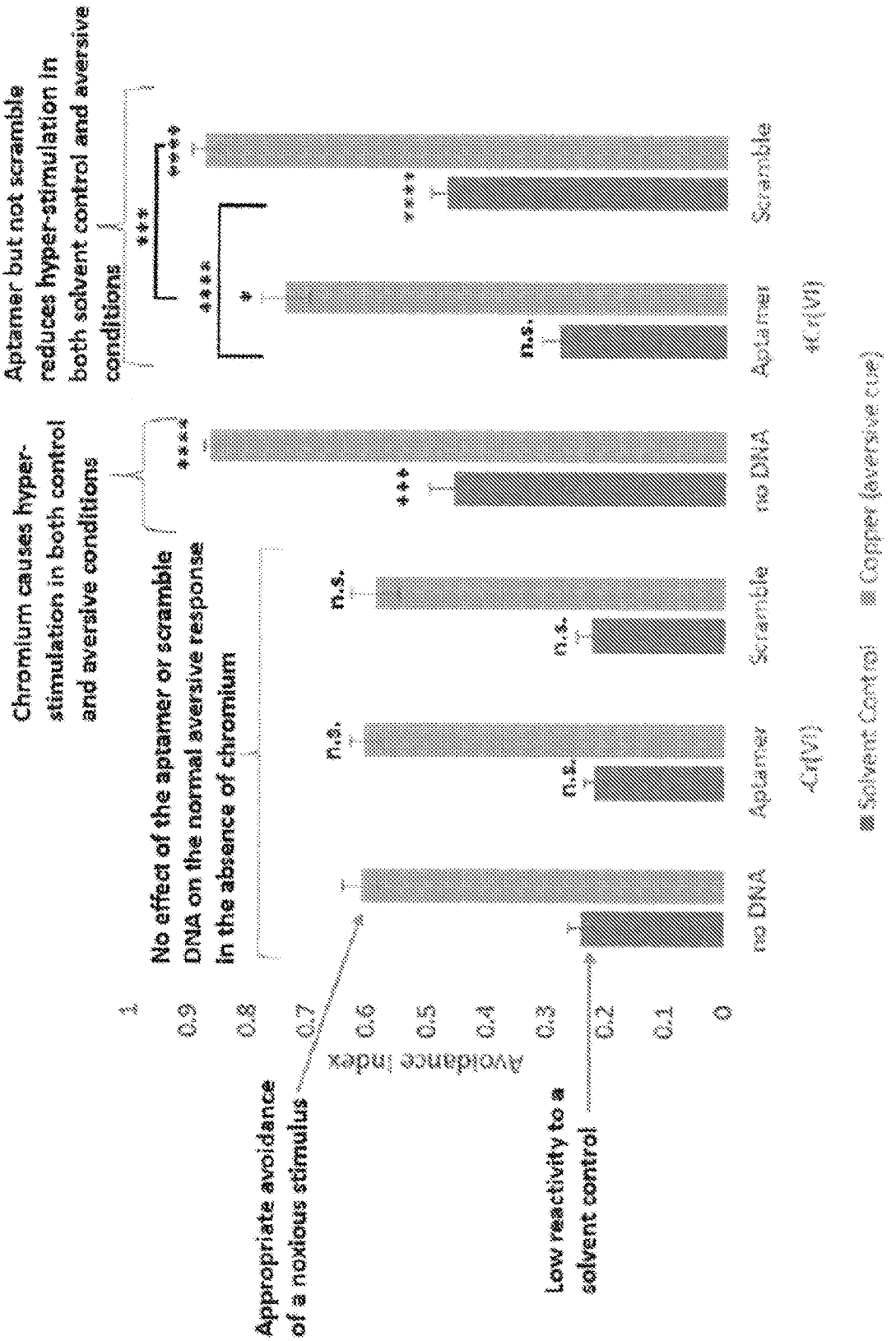

Chromium causes hyper-stimulation in both control and aversive conditions
Aptamer but not scramble reduces hyper-stimulation in both solvent control and aversive conditions
No effect of the aptamer or scramble DNA on the normal aversive response in the absence of chromium
Appropriate avoidance of a noxious stimulus
Low reactivity to a solvent control
Avoidance Index
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
n.s.
****
***
*
no DNA
Aptamer
Scramble
Cr(VI)
Solvent Control
Copper (aversive cue)

Fig. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2023/081804

A. CLASSIFICATION OF SUBJECT MATTER

A61K 48/00(2006.01)i; **A61K 31/7088**(2006.01)i; **A61P 39/04**(2006.01)i; **A61P 39/02**(2006.01)i; **C12N 15/115**(2010.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 48/00(2006.01); A61K 45/00(2006.01); A61K 9/14(2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: metal toxicity, aptamer, lead, Pb, Pb7S

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	REYES, S. R. D. A., 'Functionalized Aptamers for Lead (Pb (II)) Biosensing and Therapeutic Applications', A Thesis submitted to the faculty of Worcester Polytechnic Institute in partial fulfillment of the requirements for the Degree of Master of Science, 2022.05., pages 1-54 pages 8, 37, 40-41; table 4	1,4-13
Y		2-3
Y	LIU, H. et al., 'Structure-guided development of Pb2+-binding DNA aptamers', Scientific Reports, 2022.01.10(Online Published), Vol. 12, Article number: 460, pages 1-11 abstract; figure 1	2-3
A	FLORES-CONTRERAS, E. A. et al., `Detection of Emerging Pollutants Using Aptamer-Based Biosensors: Recent Advances, Challenges, and Outlook`, Biosensors, 2022.11.25 (online published), Vol. 12, Article number: 1078, pages 1-19 the whole document	1-13
A	HE, Z. et al., `Interfacing DNA with Gold Nanoparticles for Heavy Metal Detection`, Biosensors, 2020.11.06 (online published), Vol. 10, Article number: 167, pages 1-18 the whole document	1-13

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 April 2024	**01 April 2024**

Name and mailing address of the ISA/KR	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2023/081804

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2018-201157 A1 (WILLIAM MARSH RICE UNIVERSITY et al.) 01 November 2018 (2018-11-01) the whole document	1-13

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2023/081804

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
WO 2018-201157 A1	01 November 2018	EP 3615077 A1	04 March 2020
		JP 2020-519682 A	02 July 2020
		JP 7181282 B2	30 November 2022
		US 2020-0222453 A1	16 July 2020