Supplementary Information

Easy-to-use dipstick tests for detection of lead in paints using non-cross-linked gold nanoparticle-DNAzyme conjugates

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S1: Experimental details for lateral flow tests

a. Construction of lateral flow device

Lateral flow devices were constructed from membranes and pads obtained from Millipore Corporation, Bedford, MA. Hi-Flow Plus Cellulose Ester Membrane with a nominal capillary flow time of 240 seconds/4 cm and a nominal membrane thickness of 135 μm direct cast onto 2 mil polyester backing was used. The absorption pad and wicking pad were cut from Millipore cellulose fiber sample pads, and the conjugation pad was cut from the Millipore glass fiber conjugate pad. The absorption pad, wicking pad, and conjugation pad were attached to a plastic adhesive backing in a way shown in Fig. 1c. The overlap for each pad was ~2 mm, and the width was ~8 mm cut by a paper cutter.

b. Functionalization of gold nanoparticles with thiol modified substrate and formation of enzyme-substrate complex

Gold nanoparticles (AuNPs) with an average diameter of 13 nm were prepared by the citrate reduction method as reported in literature. Thiol modified substrate DNA was activated by adding tris-carboxy ethyl phosphine in the ratio of 1:2 (DNA : TCEP) and incubating at room temperature for 1/2 h. The gold nanoparticles were functionalized by mixing the DNA with the as prepared nanoparticles to a final DNA concentration of 3

μM and incubating for at least 16 h (Typically 9 μL of 1 mM DNA is added to 3 mL of nanoparticles). NaCl was added to a final concentration of 100 mM and the functionalized nanoparticles were ready to be used after one day incubation. DNA-functionalized nanoparticles were purified by centrifugation. To prepare the enzyme-substrate constructs, 1 mL substrate DNA-functionalized nanoparticles were purified and re-dispersed in 50 μL of buffer containing 25 mM Tris (pH 8.0), 100 mM NaCl, 8% sucrose. To this 5 μL of 1 mM enzyme was added and the sample was annealed by heating at 70 ˚C for 2 minutes and slowly cooling to room temperature over ~1 h, to obtain the hybridized construct of substrate functionalized with gold nanoparticles and enzyme (called Enz-SubAuNP). For the tests where EDTA was used, the required amount of EDTA was added to the solution before annealing.

c. Reagents application on lateral flow device

5 μL of the construct, Enz-SubAuNP was spotted on the conjugation pad. 1.5 μL of 10 mg/mL streptavidin was applied on the control zone and 1.5 μL of 1 mM capture DNA was applied on the test zone of the membrane using a 2 μL pipet. The devices were allowed to dry for 6-8 hours. After drying, the device should be stored in an air tight container with desiccant.

d. Detection.

Various concentrations of Pb^{2+} were dissolved in a flow buffer containing 25 mM Tris (pH 8.0) and 30 mM NaCl. The wicking pad of each device was dipped into the solutions for ~10 mins till the liquid had migrated to the absorption pad. Then the device was

placed flat on a plastic surface for the flow to continue for ~5 more minutes. The dipsticks were scanned when dry. For the tests in which the Pb^{2+} reaction was performed in solution, 1 µL Pb solution was added to 5 µL of Enz-SubAuNP construct and the reaction was allowed to proceed for 15 min. The reaction mixture was then applied on the conjugation pad and the devise dipped into the flow buffer. All tests were performed at room temperature (25 ˚C). The optimal working range is between 15 – 30 ˚C.

S2. Optimization of DNAzyme construct for the dipstick tests

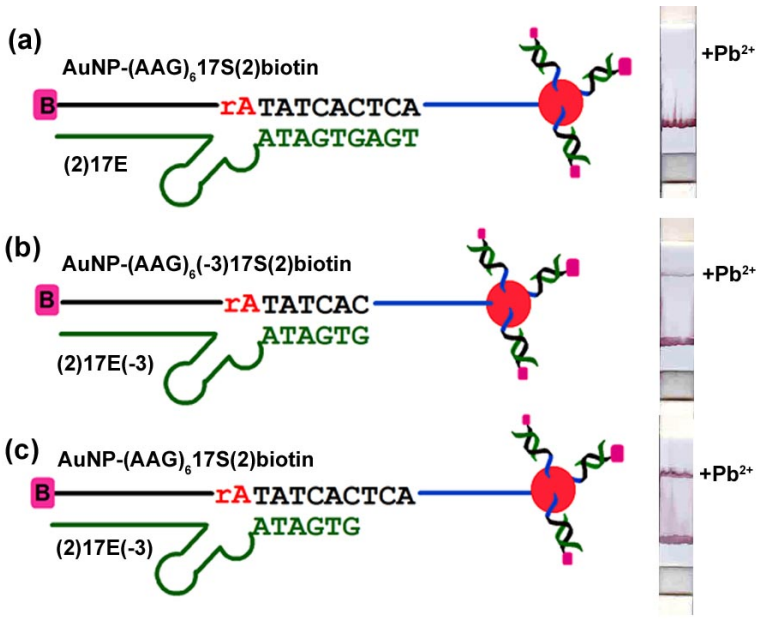
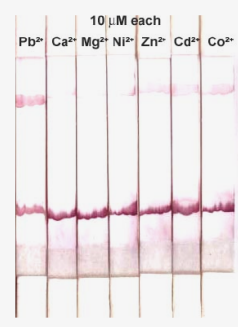


Fig. S2 Constructs used for the optimization of dipstick test. Construct (c) had the best performance in the presence of Pb^{2+} and was used for all other tests reported in this study.

To optimize the sequence of the DNAzyme construct used for the dipstick tests, one arm of the enzyme was elongated to 11 base pairs, instead of the 9 base pairs in the original 17E DNAzyme. (These bases have not been shown in the figure for clarity). The number of base pairs on the other arm (which in linked to the AuNP) was varied in order to

facilitate release. For the construct shown in Fig. S2a, there are 9 base pairs, and the dipstick stick test with Pb^{2+} doesn't show any red line at the test zone. For the construct shown in Fig. S2b, there are 6 base pairs, and the dipstick stick test with Pb^{2+} shows a faint red line at the test zone. This is because the release of the cleaved substrate piece after Pb^{2+} reaction is easier as only 6 base pairs are holding it. For the construct shown in Fig. S2b, there are 6 base pairs, however in this case only the enzyme arm is reduced in length to 6 bases whereas the substrate arm has 9 bases. The dipstick stick test with Pb^{2+} shows a dark red line at the test zone. By keeping the substrate intact, it is possible that the activity of the DNAzyme is higher as the structural perturbation is less. In addition, the capture of the cleaved piece at the test zone is better due to 3 additional base pairs with the capture DNA. Construct (c) was used in all other tests reported in this study, because its performance was the best amongst the constructs tested.

S3. Specificity



Specificity was tested at 10 µM concentration of divalent metal ions.

S4. Detection of lead in paint

a. Experimental details for detecting lead from leaded paint samples made in the laboratory

Stock leaded paints of known Pb^{2+} concentrations were prepared by mixing the white latex paint or white oil-based paint with lead (II) carbonate, basic powder (Aldrich). A 5×8 cm rectangular area was cut out in a 3M-transparency film and this was used as a template to make a rectangular paint films on pre-weighed transparency sheets by applying a layer of the stock paint and gently removing the template. The film was weighed immediately and the lead concentration was calculated. The sample was allowed to dry for at least two days. A second layer of paint was applied over this to simulate multiple paint layers in real houses.

A hole puncher was used to punch a hole of 0.283 cm^2 area and this was suspended in 0.3 mL of 10% acetic acid for ~ 3 h. The extract was separated, and diluted 10 times and adjusted to pH 5.5 using 3 M sodium acetate.

For dipstick tests, 1 µL of the extract was added to 6 µL of the construct containing 50 µM EDTA and the test was done as described in S1, part d.

b. Calculation for estimating the lead amount extracted from paint

For a 1 mg/cm^2 paint film (prepared using the method described in S3a), a spot of 0.283 cm^2 was punched for sampling

i) Pb^{2+} amount = 1 mg/cm^2 * 0.283 cm^2 = 0.283 mg

ii) Pb^{2+} in solution after extraction and dilution

= 0.283 mg/ 0.3 mL * 1/10 * 1000 mL/L ~ 90 ppm or 450 µM

iii) If 1 µL of the above Pb^{2+} solution is used in 5 µL of sensor, final Pb^{2+} concentration during reaction is 450/6 = 75 µM

iv) When 50 μM EDTA was added, it chelates 50 uM Pb^{2+} and therefore 25 μM is bioavailable for DNAzyme

For a 0.5 mg/cm^2 paint sample, Pb^{2+} in solution will be 37 μM and therefore EDTA will chelate all the Pb^{2+}