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Optical pico-biosensing of lead using plasmonic gold nanoparticles and cationic peptide based aptasensor

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Experimental details

Materials and methods

Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, N,N,N',N'-Tetramethyl-O-(benzotriazol-1-yl) uroniumtetrafluoroborate (TBTU), hydrogen tetrachloroaurate (III) trihydrate, acetonitrile, trihydrate, trisodium sodium citrate, piperidine, diethyl ether triisopropylsilane, N,N-diisopropylethylamine (DIEA), zinc sulphate, potassium bromide, mercuric chloride, cupric chloride, calcium carbonate, nickel chloride hexahydrate, cadmium chloride, sodium chloride and lead nitrate were purchased from Sigma Aldrich (India). The aptamer/oligonucleotide sequence used was 5'-$NH_2(CH)_2$-TTG GGTGGGTGGGTGGGT-3' as reported in literature.[1] The chemicals used were of analytical grade and used as such without any purification. All the reactions were carried out in ultrapure water(18.2Ω resistivity).

Synthesis of hexapeptide KRKRKR-amide

The synthesis of peptide(KRKRKR) was carried out using solid phase peptide synthesis. This procedure employs polymeric based resin and a step wise construction of peptide chain. Further, it involved repetition of alternate N-terminal deprotection and coupling reactions. Firstly, Wang resin was taken from which fluorenyl-methoxy carbonyl (Fmoc) group was deprotected using 20 % piperidine in DMF. It was followed by successive coupling reaction which employed TBTU and DIEA. The deprotection of Fmoc was carried out by using 20% piperidine in DMF after each coupling step. At the final step, the peptide was cleaved from solid support using standard protocol (95% trifluoroacetic acid/2.5% triisopropylsilane/2.5% water).The purification of the peptide was carried out using reverse phase high performance liquid chromatography (RP-HPLC) (Figure S1 and S2).

Synthesis of gold nanoparticles(AuNPs)

For the preparation of proposed aptasensorAuNPs were synthesized via chemical methodology employing $HAuCl_4$ as metal precursor and trisodium citrate as reducing as well as capping agent.[2] Briefly, aqueous solution of $HAuCl_4$ was initially heated to boiling followed by vigorous addition of 1% trisodium citrate (2 mL) to the boiled solution under continuous stirring. Initially, the color of solution appeared violet and then changed to wine red which indicated the formation of AuNPs. After the appearance of wine red color, the heating was continued for 10 minutes followed by cooling to room temperature with constant stirring. The obtained nanoparticles were purified using centrifugation and kept for storage at 4 °C. The resulting nanoparticles were then characterized using Dynamic Light Scattering (DLS) and UV-Vis spectroscopy.According to DLS results the size of prepared AuNPs found to be approximate equal to 40 nm as shown in Fig. S3.

Detection of Pb^{2+} ions

The stock solution of lead nitrate was prepared in de-ionized (DI) water and stored at room temperature. For the detection of lead ions (Pb^{2+}), firstly, 10 nM of Pb^{2+} specific aptamer (10 μL), diluted with phosphate buffer (50 μL) with pH=7.4 was incubated with different concentrations of Pb^{2+} ions (50 μL) for 40 minutes. Then 50 μM solution of cationic peptide followed by incubation for 10 minutes with sequential addition of AuNPs (200 μL). Finally, the absorption spectra were recorded using a UV-Vis spectrophotometer. In order to check the selectivity of developed aptasensor, interfering ions (Cu^{2+}, Ca^{2+}, Hg^{2+}, Zn^{2+}, Ni^{2+}, Na^{+}, Cd^{2+}, K^{+}) were tested by proposed methodology using the same procedure. Also we investigate the selectivity of lead aptamer in presence of other interfering metal ions with lead such as $Pb^{2+}$and Cd^{2+}; Pb^{2+} and Hg^{2+}; Pb^{2+} and Zn^{2+}; Pb^{2+} and Cu^{2+}; Pb^{2+} and Ca^{2+}; Pb^{2+} and Ni^{2+}. To check the practical applicability of

aptasensor, it was tested in real sample (tap water). The tap water was spiked with Pb^{2+} ions followed by detection using proposed aptasensor.

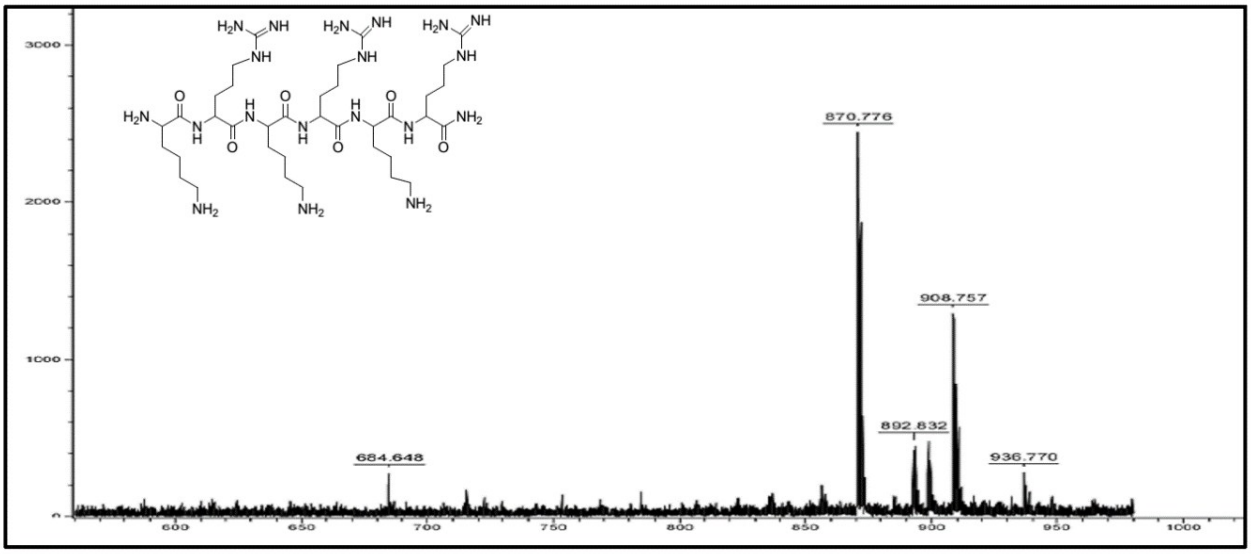
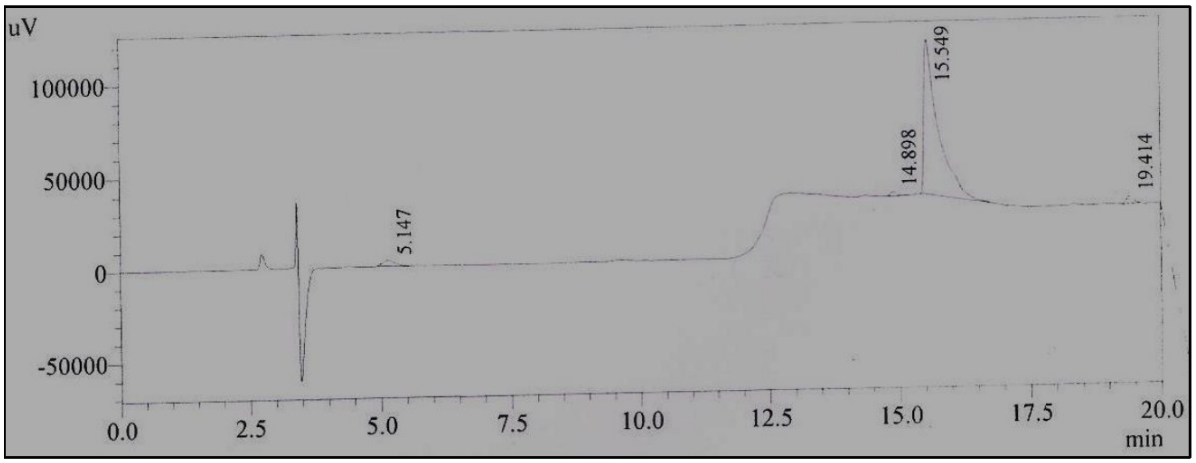


Fig.S1 MALDI-TOF spectrum of KRKRKR-amide peptide (KRKRKR-amide, MW=869 g/mol)



1 PDA Multi 2 / 215nm 4nm

PeakTable

PDA Ch2 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	5.147	47783	2983	2.906
2	14.898	11938	2076	0.726
3	15.549	1550264	82567	94.272
4	19.414	34472	3824	2.096
Total		1644457	91449	100.000

Fig. S2 HPLC chromatogram of synthesized cationic peptide KRKRKR-amide

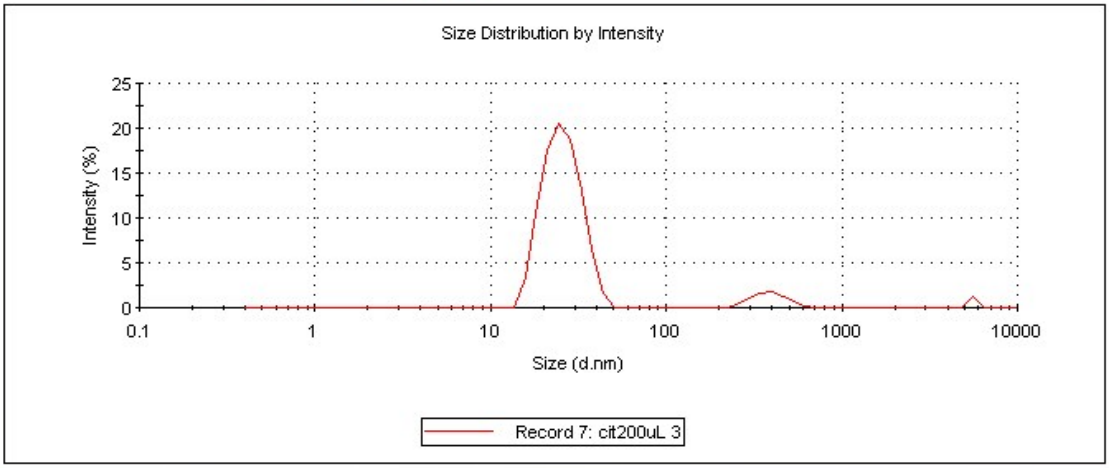


Fig. S3 DLS results for the synthesized Gold nanoparticles (Size≈ 40 nm)

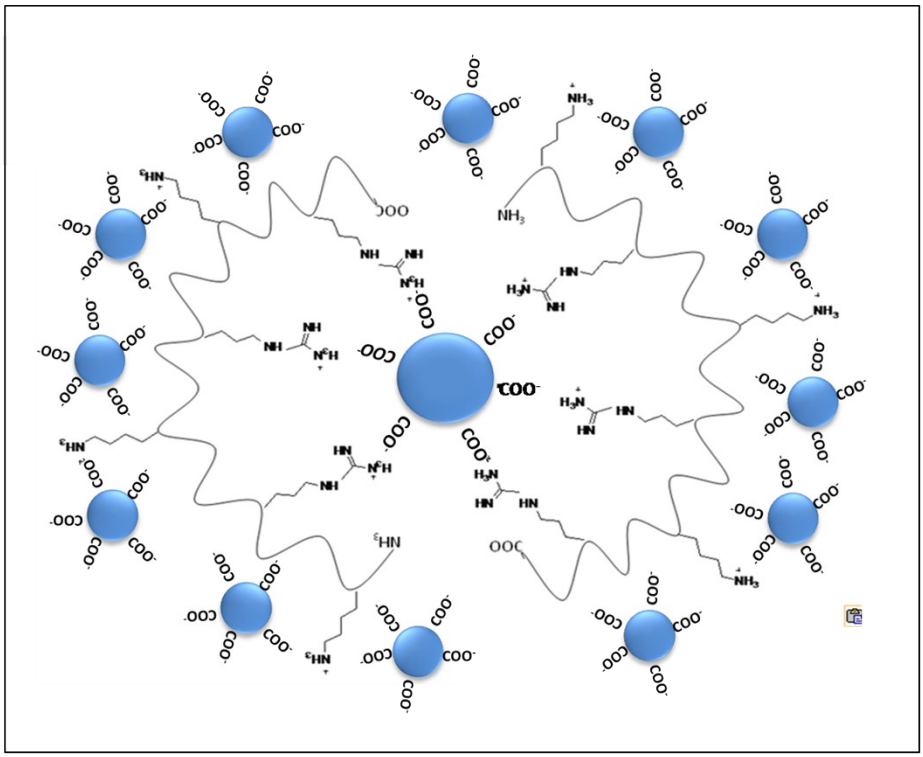


Fig. S4 Probable Interactions between gold nanoparticles and KRKRKR peptide(aggregated gold nanoparticles)

Interactions between Peptide and AuNPs-

Citrate capped gold nanoparticles are highly negatively charged at physiological pH due to the presence of carboxylic group on their surface. Due to this, negative charge on their surface AuNPs repel each other and remain well dispersed in the solution with red color. Peptide KRKRKR which is used in this scheme carries lysine(K) and arginine(R) amino acids. This hexapeptide is highly positively charged due to guanidinium moiety in argnine and amino group in lysine side chain. Guanidinium moiety and amino group in peptide interact electrostatically with carboxylic group of AuNPs. As a result, the nanoparticles come close to each other resulting in the aggregation of AuNPs. This aggregation changes the color of AuNPs from red to blue and also absorption peak undergoes red shift.

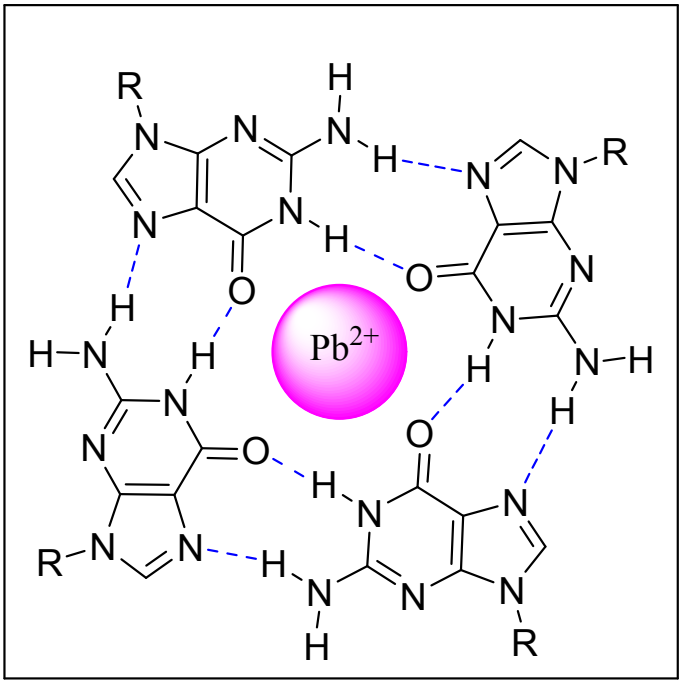


Fig.S5 Probable molecular interactions between Pb^{2+} and apatmer bases.

Interactions between aptamer and metal ions-

The target metal ion (Pb^{2+}) interacts with the single strand DNA (aptamer) through mainly two types of molecular interactions:

(a) Electrostatic interactions

(b) Quadruplex interactions.

Pb^{2+} ions interact with both phosphate backbone and nucleobases of aptamer.Each phosphate in phosphate backbone of aptamer contains one negative charge with a pKa below 2 and due to this, aptamers are highly negatively charged at physiological pH.[3] Aptamers are higly stable due to electrostatic repulsions between phosphate groups. These repulsions can be disturbed by Pb^{2+} ions. Pb^{2+} ions electrostatically interact with negatively charged oxygen atoms of the phosphate and also it coordinates with selective nucleobases (e.g., N3 and O2 of cytosine, N7 and O6 of guanine, and O4 of uracil) through their electron rich sites.[3] Nucleotides act as metal ligandswhen metal shows binding affinity towards some specific nucleobases. Nucleotide coordination affinity towards Pb^{2+}ions is given below.[4]

Metal Ion	Phosphate	Adenine N7	Guanine N7 and O6	Cytosine N3 and O2
Pb^{2+}	1.5	0.9	1.75	1.65

Guanine rich aptamer of Pb^{2+} ion can form guanine tetrad when guanine bases interact with lead ions and two or more guanine tetrad stack on each other and form G-quadruplex structure resulting in the folding of aptamer(Apt-Pb^{2+}).[5,6]

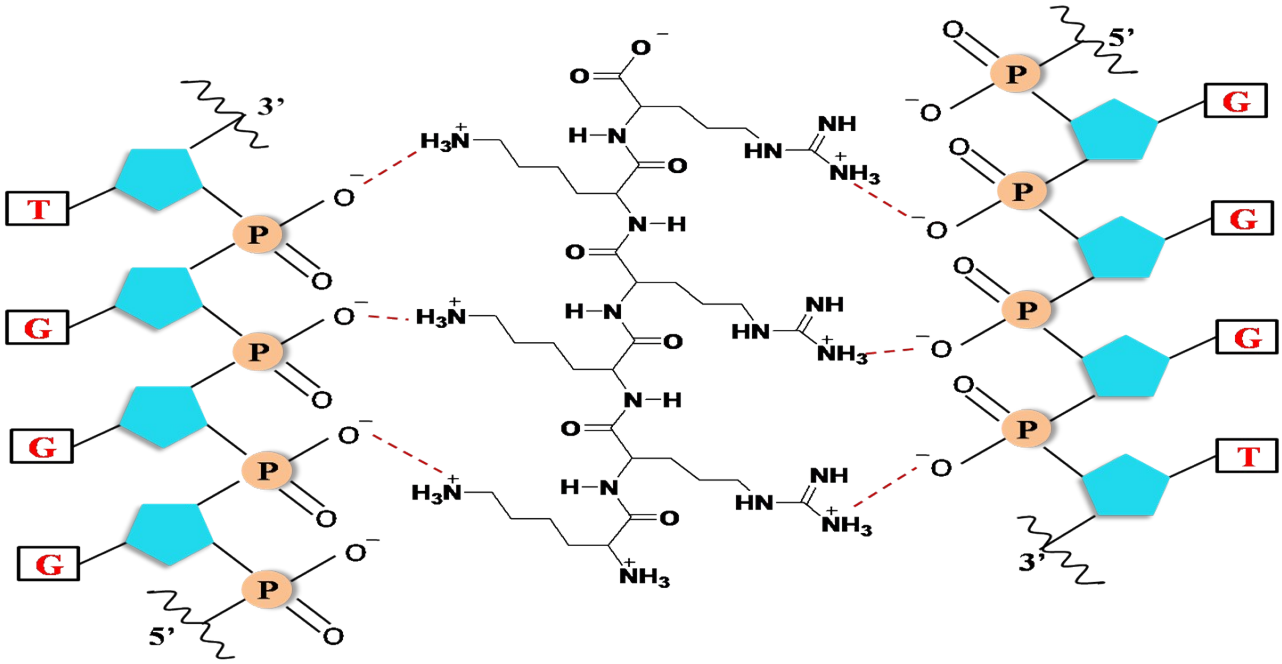


Fig. S6 Probable molecular interactions between aptamer and peptide

Interactions between Aptamer/pepetide duplex:

Aptamer and peptide interact with each other via electrostatic interactions between the positively charged peptide and negatively charged aptamer.[7] Phosphate backbone of aptamer is negatively charged at physiological pH. Peptide KRKRKR which is used in this scheme carries lysine(K) and arginine(R) amino acids which combine to each other alternatively. This hexapeptide is highly positively charged due to guanidinium moiety in argnine and amino group in lysine side chain. Guanidinium moiety in arginine and amino group in lysine side chain possess pKa 12.58 and 10.53 respectively, which keeps themprotonated at physiological pH.[8] Negatively charged oxygen of phosphate in apatmer electrostatically interacts with positively charged amine groups in the hexapeptide as shown below.

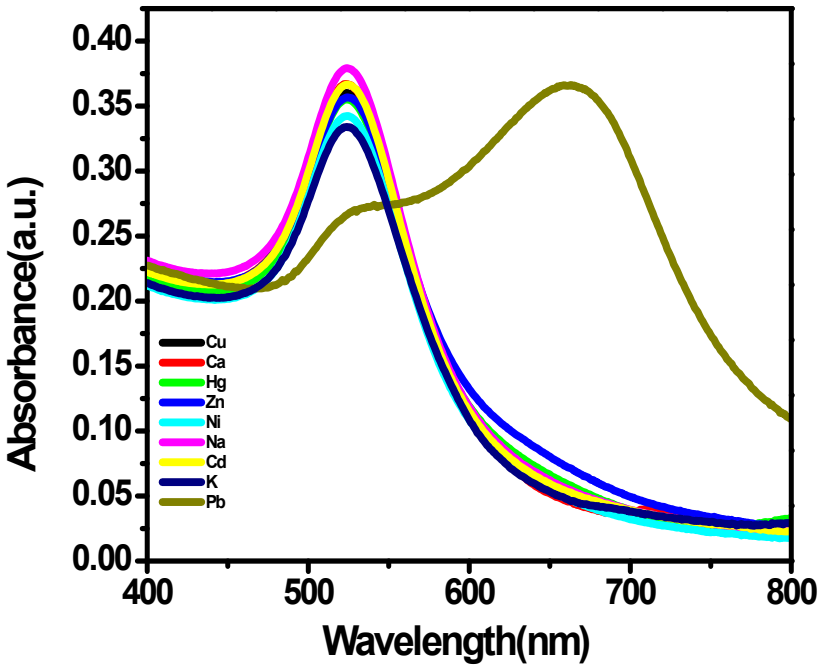


Fig. S7 Absorbance spectra depicting the sensor array response to tested ions. The concentration of tested metal ions was 10 mM and Pb^{2+}ions was 1mM. Inset the picture showing the aptasensor's selectivity for Pb^{2+} ions.

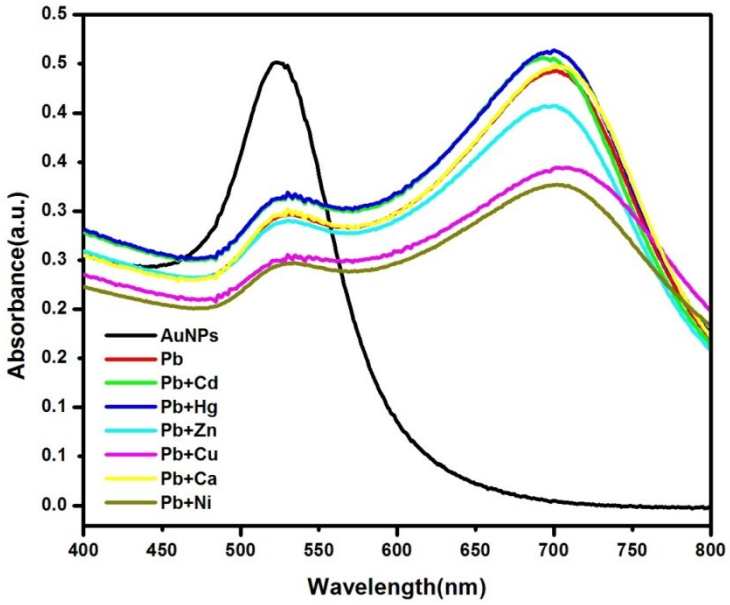


Fig. S8 Absorbance spectra depicting the sensor array response to tested ions with lead ions. The concentration of tested metal ions was 1 μM.

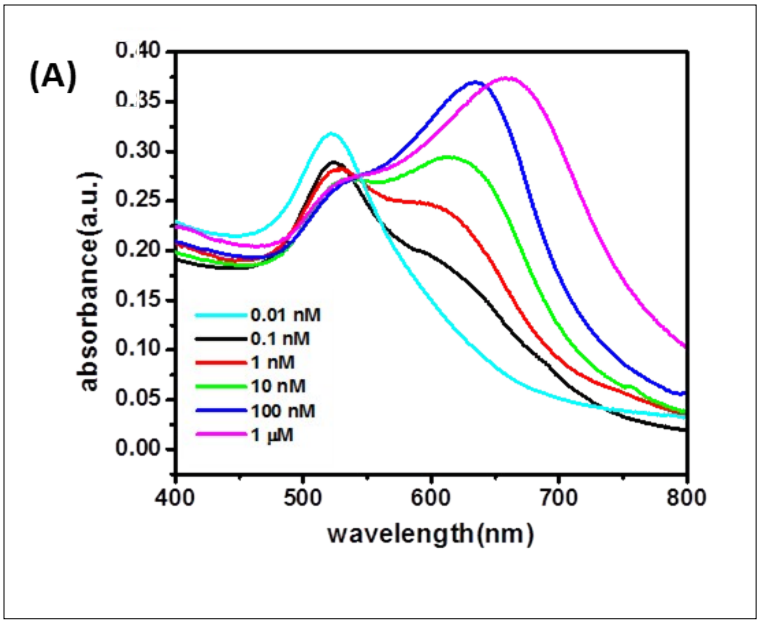


Fig.S9 (A) Absorbance spectra of AuNPs having different concentration of lead ions in mineral water. The lead ions are not present in mineral water, so spiking was done. The peptide and aptamer concentration for reaction are 50 μM and 10 nM.

Fig. S10 Absorbance spectra depicting the reproducibility of biosensor

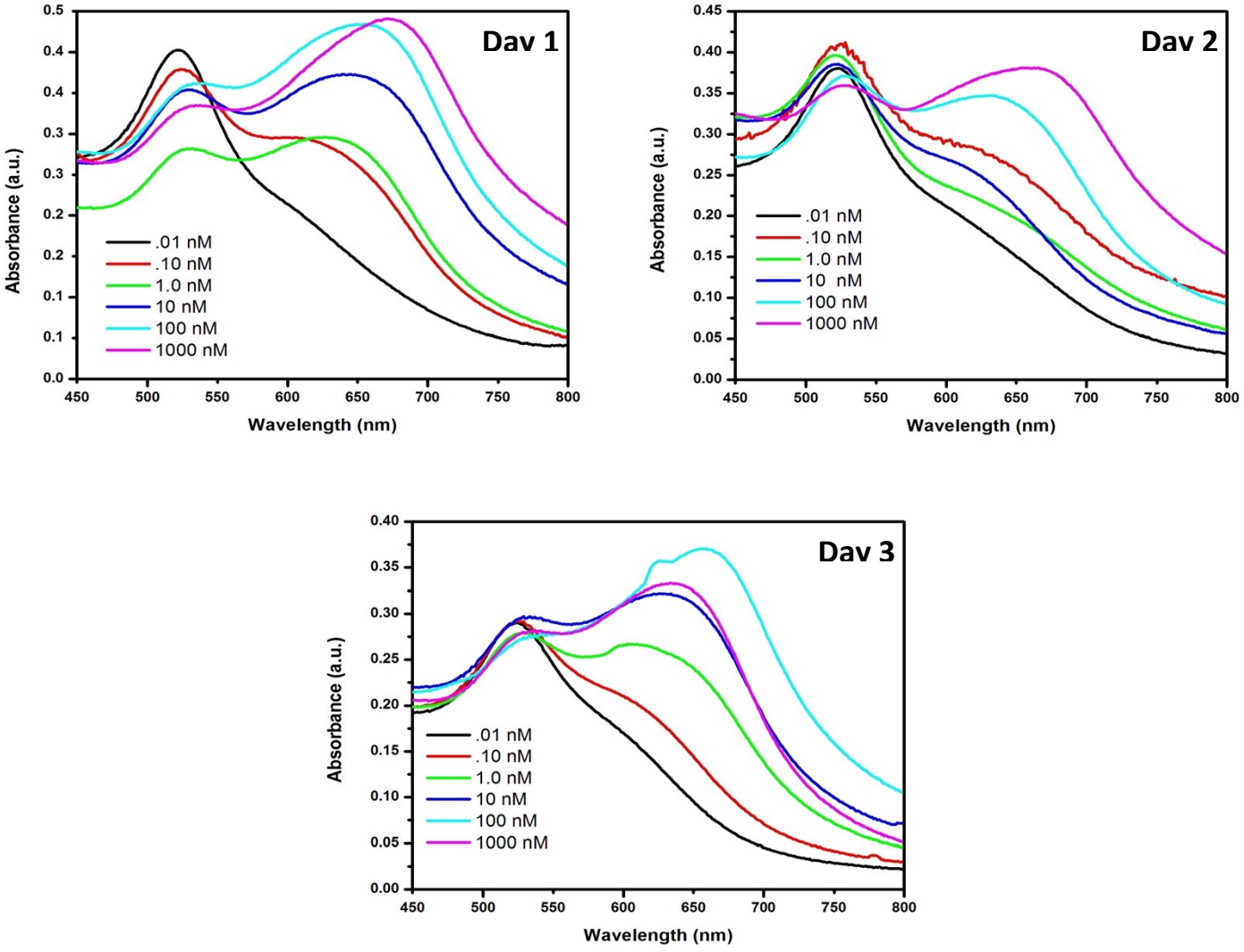


Table S1 Analogy of lead ions concentration in tap water employing devised aptasensor.

Spiked amount (nM)	Amount Calculated (nM)	Recovery (%)
0.01	0.009	90
1	1.006	100.6

Calculation S1Limit of Detection(LOD)

LOD = 3α/m

α = Standard deviation
m = slope
LOD = (3 × 0.002654)/0.0805
LOD = 98.7pM

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