Supplementary material of paper entitled: **Functionalized aptamers as nano-bioprobe for ultrasensitive detection of Bisphenol-A (ID CC-COM-03-2013-042002)** authored by Vasanth Ragavan K, Selvakumar L. S and Thakur M. S * submitted to your esteemed journal Chemical communications.

Chemicals and reagents

DNA aptamer for Bisphenol A and scrambled DNA aptamer were obtained from Custom Oligos, Bangalore, India. Functionalized BPA aptamer tagged with FAM and BHQ-1 at 3' and 5' ends were procured from Trilink Biotechnologies, San Diego, USA. Tetrachloroauricacid, sodium citrate tribasic anhydride, silver nitrate, bisphenol-A, dimethylsulfoxide (DMSO) and bisphenol analogues were procured from Sigma Aldrich Chemicals, USA. Salts of different metal ions and sodium chloride were procured from Qualigens Fine Chemicals, India. Milli-Q water from Millipore-Elix equipment was used to prepare the reagents. A stock solution of BPA was prepared by dissolving 1mg of BPA in BPA binding buffer (25mM TrisHCl, 100mM NaCl, 25mM KCl, 10mM $MgCl_2$, 5%DMSO, pH8). A stock solution of 100µMDNA aptamer and functionalized aptamer as well as further dilutions was prepared in BPA binding buffer. 1M NaCl solution and metal ion solutions were prepared in double distilled water and stored at 4^oC till further use.

Aptamer Sequence:

5'-CCGGTGGGTGGTCAGGTGGGATAGCGTTCCGCGTATGGCCCAGCGCATCACGGG TTCGCACCA-3'

Functionalized aptamer sequence:

BHQ 5'-CCGGTGGGTGGTCAGGTGGGATAGCGTTCCGCGTATGGCCCAGCGCATCACG GGTTCGCACCA-3' FAM

Scrambled Aptamer Sequence:

BHQ 5'-AAGCTGGGATGGCATGCGTACTCCCCAGACACAACCGGCCAAGTACATGACAC CATTCACAAA-3' FAM

Apparatus

Spectral analysis of GNPs was carried out using spectrophotometer UV-1601 (Shimadzu, Japan). The fluorescence emission spectrum of the functionalized aptamer was obtained using Shimadzu RF-5301 PC spectrofluorometer (Japan). Circular dichroism spectrometry was carried out using Jasco, J-810 spectropolarimeter.

GNPs Synthesis

An aqueous solution of monodisperse quasi-spherical GNPs was synthesized by modified Turkevitch et al., method.[1,2] For GNPs synthesis, 45 mL of Milli-Q water was taken in a reaction flask and refluxed for 10 min with 5 mL of 0.1% tetrachloroauricacid ($HAuCl_4$), 2 mL of 1% trisodium citrate and 42.5 µL of 0.1% of silver nitrate. Tetrachloroauricacid, trisodium citrate and silver nitrate were mixed together in a separate beaker and incubated for 5 min and added in drops to the reaction flask. The reduction of gold ions (Au^{3+}) to yield GNPs (Au^{o}) was apparent from the appearance of dark cherry red color. Colloidal GNPs was stored at 4°C till further use. All the glasswares used for the GNPs synthesis were washed with aqua regia followed by rinsing with double distilled water and dried in hot air oven. The spectral characteristics of synthesized GNPs were obtained which has a surface plasmon resonance peak at 515nm. Using the absorption spectra of GNP the size and concentration of GNPs was calculated following Haiss et al., method.[3] Average size of GNPs was found to be 11nm which correlates well with the TEM characterization.

Optimization Studies:

Initially the parameters which control the assay are optimized to achieve higher sensitivity and performance. Concentration of aptamer was optimized by taking different concentrations of aptamer in the range of 0.25µM to 1µM with constant amount of GNPs and salt concentration and the spectra were taken in the wavelength range of 400-700nm using Shimadzu UV-1602 PC spectrophotometer (Japan) (Fig.S1). Volume of GNPs was optimized by taking different volumes of GNPs in the range of 50µL to 400 µL along with constant amount of aptamer and salt in the assay and the spectra were noted in the range of 400-700nm (Fig.S2). Respectively different concentrations of salt in the range of 0.1M to 2M were taken with constant amount of aptamer and GNPs and the spectra in the above mentioned range were noted down (Fig.S3). Finally the incubation time for performing the assay was done by incubating the assay for different period of time in the range of 0min to 60 min and the spectra were noted down. For the analysis of BPA, we took the optimized concentrations of all parameters from their respective spectra and performed the assay. The change in color was perceived visually and spectra of the solutions were observed using spectrophotometer. The concentration of functionalized aptamer was optimized for

fluorescence emission after the binding of BPA with functionalized aptamer. We took different concentrations of 200µL of functionalized aptamer in the range 0.1µM to 0.01 µM and excited at a wavelength of 496nm and emission at 520nm were noted. After an incubation time of 5min we took the spectra of the conjugates with the above said parameters and the spectra were plotted as shown in Fig-S4.

Binding and cross reactivity

In order to study the specificity of the BPA aptamer towards BPA and its analogues we did a cross reactivity study. In the assay we took 100ng/mL of BPA and its analogues like BPB (2,2-Bis(4-hydroxyphenyl)butane), BPAF (2,2-Bis(4-hydroxyphenyl)hexafluoropropane) and BPS (Bis(4-hydroxyphenyl)sulfone). By following the optimized parameters we performed the assay and the absorbance spectra was noted. In case of functionalized aptamer, BPA and its analogues were taken in a concentration of 10pg/mL and added to aptamer concentration of 0.8µM and the emission spectrum was noted with an excitation at 496nm.

Scrambled aptamer assay

To confirm the specificity of aptamer towards BPA scrambled DNA aptamer of same length was tagged with FAM and BHQ. The scrambled aptamer has completely different sequence compared to DNA aptamer of BPA. BPA concentration of 10pg/mL was added with 0.1µM scrambled aptamer and incubated for 5 min. With an excitation at 496nm and emission at 520nm the spectra was noted which showed insignificant change in the fluorescence emission (Fig.S5). The result clearly indicates the scrambled DNA aptamer is insensitive towards BPA and further confirmed by performing CD spectroscopy to study the conformational changes during the assay.

Circular Dichorism (CD) Studies

CD spectroscopic studies were performed to examine any conformational changes in the scrambled DNA aptamer with BPA. The assay was performed by taking 5µM of the scrambled DNA aptamer with 10pg/mL of BPA in a 1cm path length quartz cuvette using Jacso-810 spectropolarimeter. The CD spectra of scrambled aptamer display too little change in the conformation with BPA when compared to native scrambled aptamer thereby confirming its inability to bind BPA (Fig.S6). The results correlate well with the scrambled aptamer assay and confirm the selected DNA aptamer sequence is specific towards BPA.

Real Sample Analysis:

To check the applicability of the proposed work, urine samples were taken and the amount of BPA present in the sample was analyzed using the proposed method. From the assay, the fluorescence remained same and it didn't "turned-off" which interprets the absence of BPA. Generally in biological samples BPA is found in conjugated forms (BPA-glucronide, BPA-disulphate and BPA-chloride) [4] and the aptamer is not specific to the conjugated forms. So, we spiked the same urine samples with different concentration of BPA and performed the assay. The results showed decrease in fluorescence ("turn-off") proportional to the amount of BPA in urine sample (Table.S1).

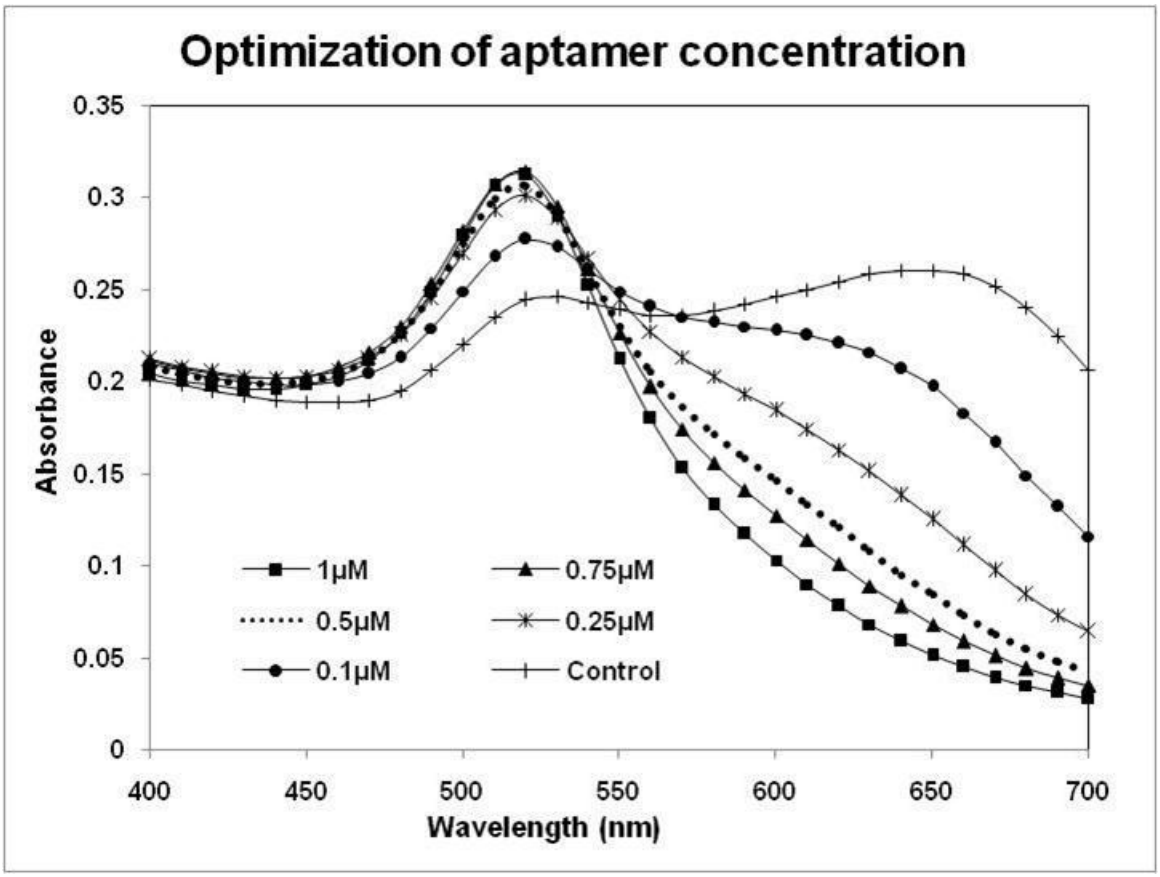

Fig-S1: Absorption spectra for optimization of aptamer concentration.

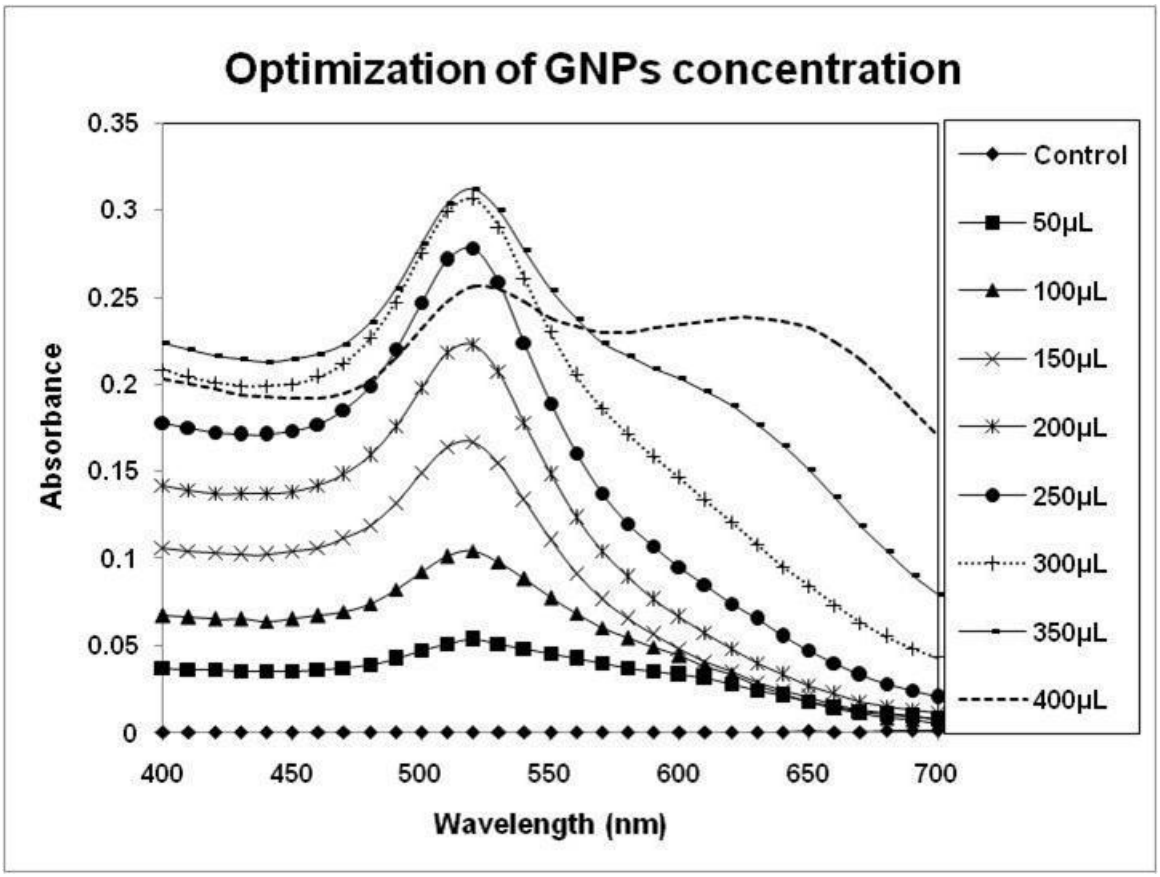

Fig.S2: Absorption spectra for optimization of GNPs.

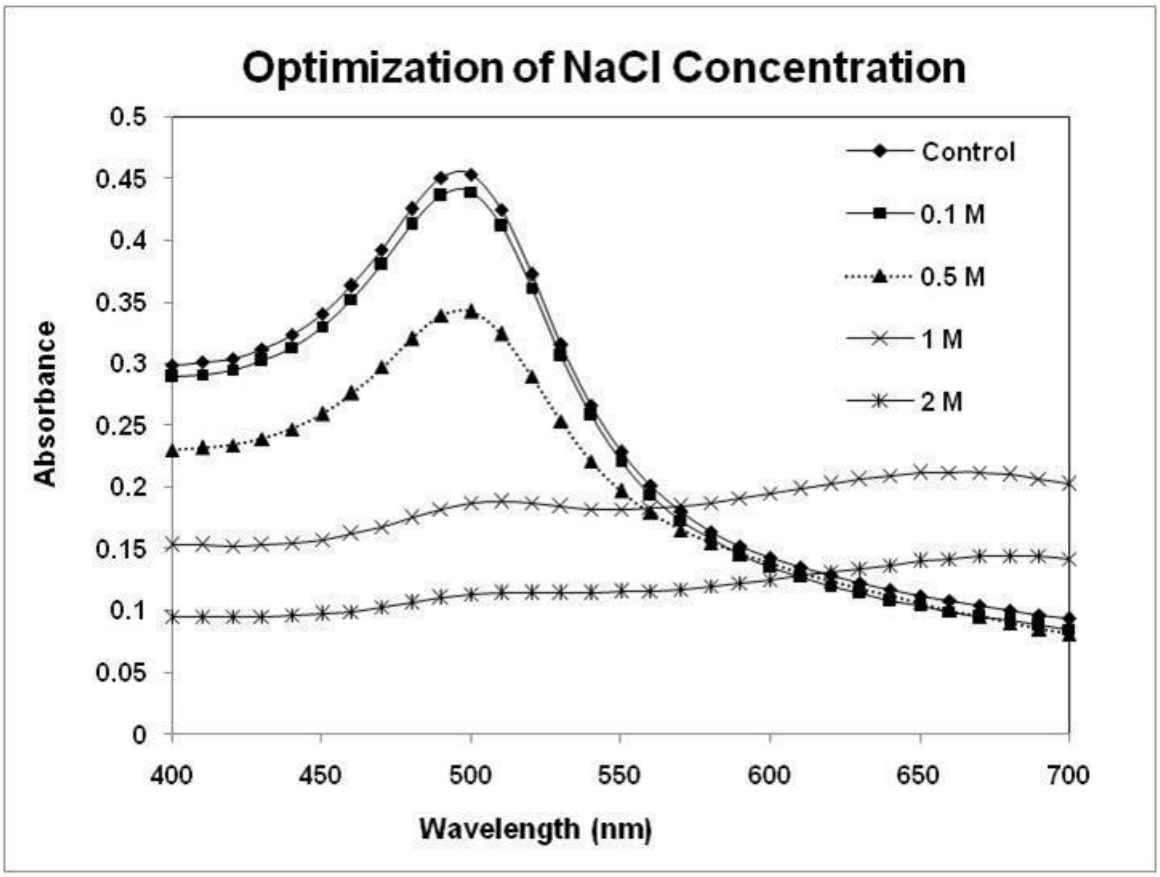

Fig.S3: Absorption spectra for optimization of NaCl concentration.

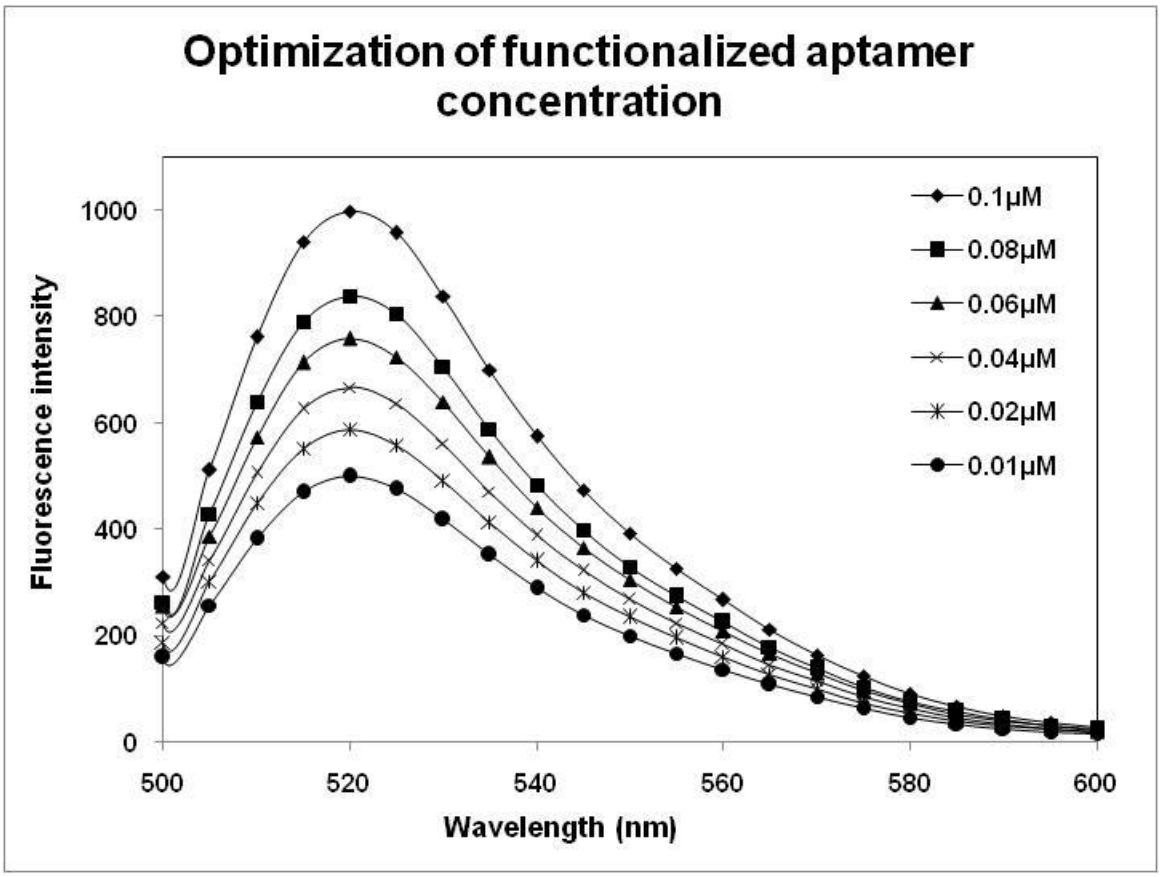

Fig.S4: Optimization of functionalized aptamer concentration.

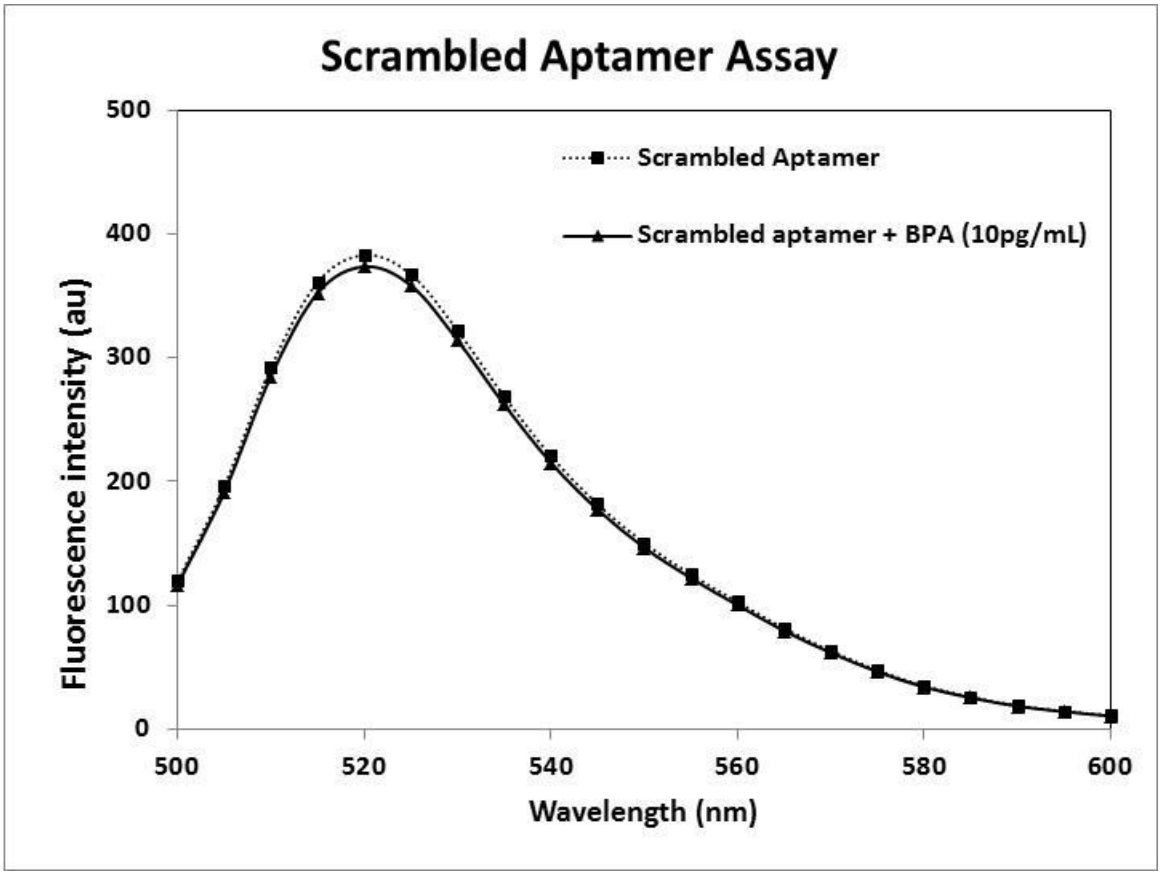

Fig.S5: Scrambled aptamer assay.

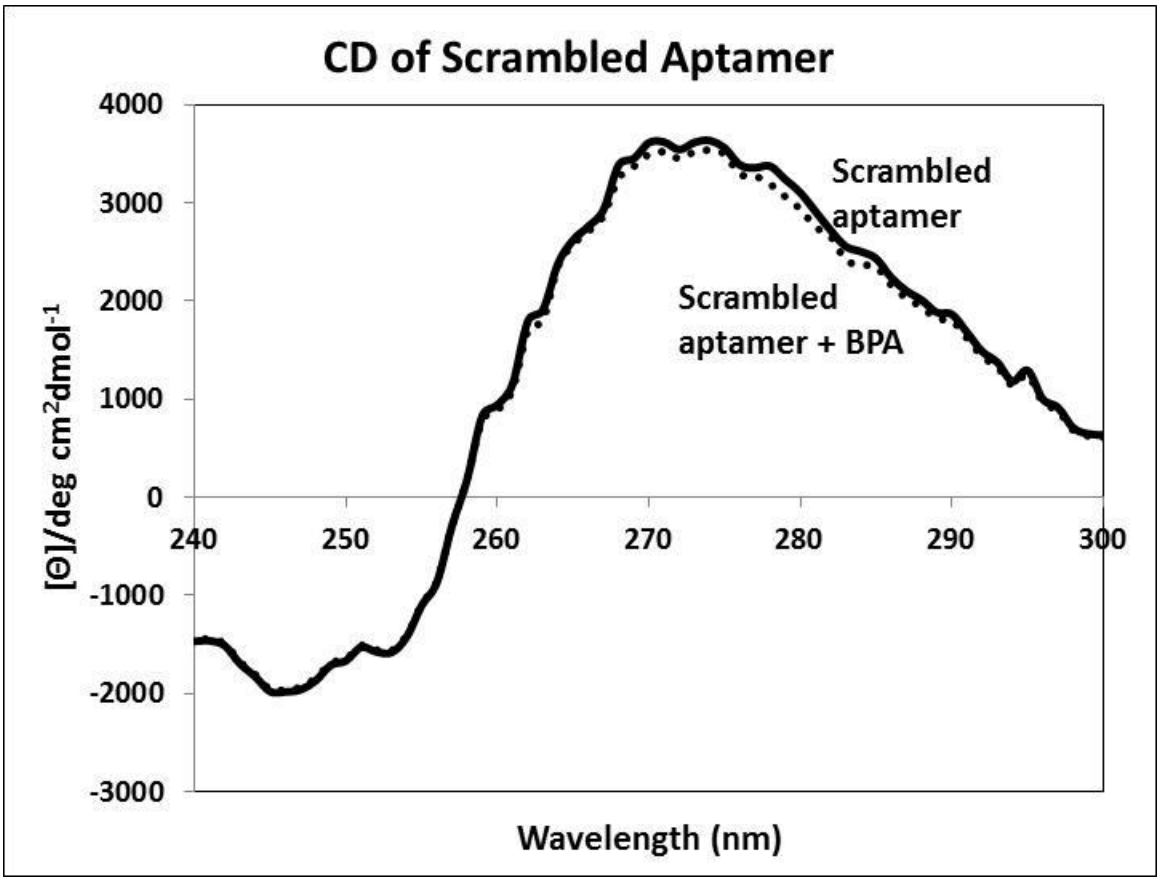

Fig.S6: CD spectra of scrambled aptamer and in presence of BPA.

Urine Samples	Amount fortified (pg/mL)	Amount found (pg/mL)	
		Aptamer-GNPs method	Functionalized aptamer method
Sample-1	0	Not Detected (ND)	ND
	0.01	ND	ND
	0.1	ND	0.0818± 0.0053
	1	ND	0.926± 0.040
	10	ND	10.042± 0.65
	100	78.2±0.53	98.26± 5.085
	1000	952.8±0.03	990.4± 24.8
Sample-2	0	ND	ND
	0.01	ND	ND
	0.1	ND	0.0844± 0.015
	1	ND	0.9458± 0.055
	10	ND	9.648± 0.42
	100	81.6±0.39	100.3± 3.9
	1000	978.2±0.78	1001.2 ± 40.37
Sample-3	0	ND	ND
	0.01	ND	ND
	0.1	ND	0.0852± 0.008
	1	ND	0.96± 0.07
	10	ND	9.886± 0.33
	100	101.6±0.50	100.16± 6.88
	1000	971.7±0.032	1031.8± 60.85

Table.S1: Analysis of BPA in urine samples using aptamer-GNPs assay and compared with functionalized aptamer assay. Amount of BPA fortified in real samples is 1000pg/mL to 0.01pg/mL.

References:

1. J. Turkevitch, P.C. Stevenson and J. Hillier, Faraday Society, 1951, **11**, 55-75.

2. H. Xia, S. Bai, J. Artman and D. Wang, Langmuir, 2010, **26**, 3585-3589.

3. W. Haiss, N.T.K. Thanh, J. Aveyard and D.G. Fernig, Anal Chem, 2007, **79**, 4215-4221.

4. C. Liao and K. Kannan. Environ. Sci. Technol. 2012, **46**, 5003−5009.