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Functionalized aptamers as nano-bioprobes for ultrasensitive detection of bisphenol-A†

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A novel functionalized aptamer based 'turn-off' fluorescent biosensor for ultra-sensitive detection of small molecules like bisphenol-A in water samples.

Aptamers are synthetic single stranded nucleic acids which recognize a wide range of compounds from ions to cells. Aptamers undergo conformational changes after binding with the target.[1,2] Aptamers are selected *in vitro* from a large library of nucleic acid sequences (10^{15}) by the process known as systematic evolution of ligands by exponential enrichment (SELEX).[3] Compared to antibodies, aptamers are highly specific, selective, stable over a wide range of temperatures, can be stored for long durations and economical, hence they are increasingly used as recognition elements in biosensors.[4] Aptamers play a significant role in the detection of small molecules at very low concentrations.[5–10]

Gold nanoparticles (GNPs) are used as a color indicator in biosensing assays due to their excellent physical, optical and distance dependent properties. GNPs exhibit a strong absorption band in the visible region due to their particle size. Small GNPs (10–50 nm) are ruby red in color due to localized surface plasmon resonance (LSPR), but the aggregated GNPs are blue in color with a shift in absorption towards the red end of the visible spectrum.[11]

We have chosen bisphenol-A (BPA) as the target molecule for proving the principle. BPA is an endocrine disrupting compound and humans are widely exposed to this compound.[12] A DNA aptamer specific to bisphenol-A was reported by Jo and his coworkers.[13] An aptamer–gold nanoparticle (GNP) based system for BPA detection was reported by Mei and his group.[14] Since the detection is based on various parameters of the assay the detection becomes a complex process. We propose a functionalized BPA aptamer based 'turn-off' biosensor. A change in the aptamer conformation during the binding of the target is exploited for the detection of BPA. Therefore the detection is direct and independent of other assay parameters. So, we functionalized the DNA aptamer of BPA by tagging FAM (fluorescein amidite) and BHQ-1 (black hole quencher) at 3′ and 5′ ends respectively. The DNA aptamer of BPA is rich in guanine residues at the 5′ end which are naturally fluorescence quenchers and if FAM is attached at the 5′ end the emission of FAM will be quenched by guanine residues and finally reduced. So we attached FAM at the 3′ end, as a result it exhibited unhindered emission as well as the quenching efficiency of BHQ was enhanced due to the presence of guanine residues at the 5′ end, and sensitive detection is possible with this modification.[15] Emission spectra of FAM overlap with the absorption spectra of BHQ, provided the molecules are close enough within the Förster radius. The functionalized aptamer in its native state has high fluorescence owing to the aptamer ends being apart. After binding with BPA the functionalized aptamer undergoes an 'induced fit' binding mechanism and the ends come closer.[16] Due to spectral overlapping the emitted energy of FAM is quenched by BHQ within the Förster radius owing to the energy transfer phenomenon. There will be a gradual reduction in the emission with increase in BPA concentration of the solution. Hence binding of BPA with the functionalized aptamer reduces the fluorescence emission ('turn-off') of the solution. Earlier the functionalized aptamer mechanism was used for designing 'turn-on' biosensors for the detection of ATP and thrombin.[17,18] We compared both aptamer and functionalized aptamer based assays ('turn-off') for the first time for the detection of BPA. The sensitivity of this method is far better compared to available analytical and aptamer based methods.

The working principles of the aptamer based assay are shown in Fig. 1. In the case of the aptamer–GNPs method aptamers adsorb on the GNPs surface due to electrostatic interaction between them. The complex is stable upon addition of a salt and remains red in colour.

Aptamers being specific to BPA will desorb from the GNPs surface and bind to BPA, thereby leaving the GNPs surface unprotected. With the addition of a salt, the unprotected GNPs get aggregated due to the salt induced 'non-crosslinking mechanism' and the colour turns blue.[19] In the case of the functionalized aptamer, the native and unbound aptamer has maximum fluorescence, whereas upon binding with BPA the fluorescence emission of the aptamer decreases leading to 'turn-off' of emitted energy. The proposed 'turn-off' biosensor is much simpler and involves a single step detection

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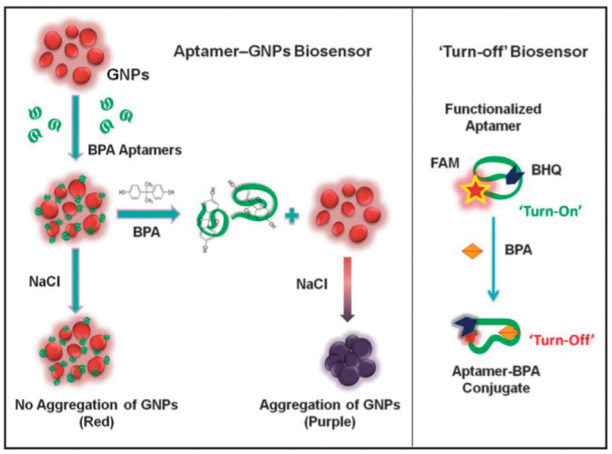

Fig. 1 Scheme of functionalized aptamer based methods for the detection of BPA; (a) aptamer–GNPs based assay and (b) 'turn-off' biosensor.

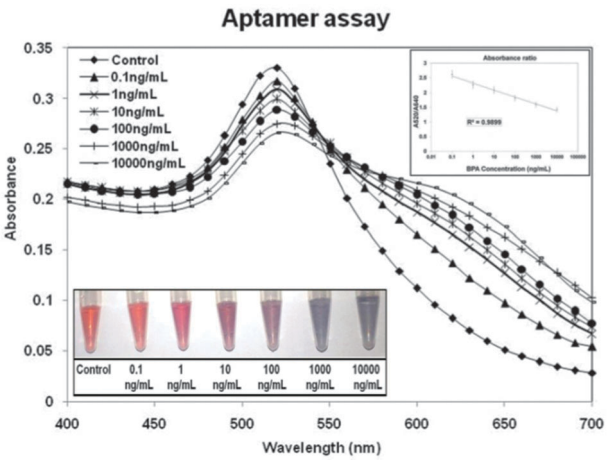

Fig. 3 Absorption spectra of aptamer–GNPs assay. Inset images show the change in colour of GNPs during the assay and the plot of A_{520}/A_{640}.

method compared to the aptamer–GNPs method. The 'turn-off' bio sensing method can be successfully applied to small molecules since the detection is very straightforward.

GNPs and the functionalized aptamer were characterized and their respective spectra are shown in Fig. 2. GNPs in the absence of a target show a characteristic surface plasmon resonance peak around 520 nm, whereas in the presence of BPA the GNPs get aggregated and turn blue indicated by broadening and shifting of the peak towards the red end of the spectrum.

In the case of the functionalized aptamer we observed characteristic emission spectra of FAM at 520 nm indicating that the aptamer is in its native state devoid of BPA. For the analysis of BPA, assay parameters like aptamer concentration, GNPs concentration, salt concentration and incubation time were optimized to achieve maximum detection limits for BPA. We found 100 μL of 0.5 μM aptamer, 300 μL of GNPs and 50 μL of 0.5 M NaCl to be optimal. The optimized conditions of assay parameters were used for the detection of BPA in water samples. The change in color was perceived visually and spectra of the solutions were observed using a spectrophotometer. With high concentrations of BPA in solution, the color of GNPs turns purple and we observed a gradual change of the color from red to purple with increasing BPA concentration. Even though at lower concentrations the color change is not prominent to differentiate visually, spectra show a substantial difference in the SPR peak as shown in Fig. 3. The limit of detection using this method was 0.1 ng mL^{-1} with linearity in the range 10 000 ng mL^{-1} to 1 ng mL^{-1}.

In the case of 'turn-off' assay 200 μL (0.08 μM) of the functionalized aptamer was taken with various concentrations of BPA in the range of 0.01 pg mL^{-1} to 100 000 pg mL^{-1} in solution. After an incubation time of 5 min the spectra of the solution with excitation at 496 nm and emission at 520 nm were recorded. The functionalized aptamer in the absence of BPA in solution had maximum fluorescence due to its native conformation, whereas in the presence of BPA the fluorescence emission of the aptamer drops to a lower level. The functionalized aptamer being very specific to BPA undergoes binding by the induced fit binding mechanism and leads to a change in the structural conformation of the aptamer. The structural change of the aptamer brings the ends together much below the Förster radius where the emission of the FAM molecule at the 3′ end lies in the absorption range of BHQ at the 5′ end. The energy from FAM is transferred to BHQ and is quenched, therefore the final emission decreases with the binding of BPA. We observed a gradual decrease in the fluorescence emission with a linear increase in the concentration of BPA from Fig. 4. The detection was linear in the range 10 000 pg mL^{-1} to 0.1 pg mL^{-1} with a detection limit of 0.01 pg mL^{-1}.

The obtained results gave a hint on the structure of the BPA aptamer based on binding with its target. The change in structure acts as a 'turn-off' model for the detection of BPA which was exploited for designing the biosensor for BPA. Changes in structural conformation of the aptamer from its native state to bound state were studied using circular dichroism spectra of the aptamer and aptamer–BPA. The spectra clearly indicate that aptamer–BPA has different spectra compared with the aptamer, which indicates that the aptamer undergoes conformational changes after binding with the target as shown in Fig. 5.

Selectivity of the BPA aptamer was studied by performing cross-reactivity studies using BPA analogs. During the assay only the sample containing BPA has aggregated, in the case of analogues there was no aggregation indicating that the aptamer has not desorbed from the GNPs and protects them from salt induced aggregation. The above assay shows that the selected aptamer is highly specific to BPA and has insignificant cross-reactivity with its analogs.

The same pattern was observed for the emission spectrum in functionalized aptamer cross-reactivity studies. There was no considerable reduction of emission in the case of BPA analogues, whereas BPA showed a good reduction in emission (as shown in Fig. 6).

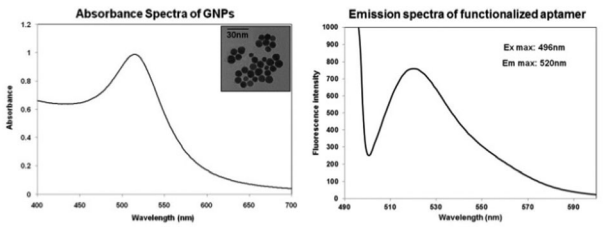

Fig. 2 (a) Absorption spectra of GNPs with the inset showing a TEM image and (b) emission spectra of the functionalized aptamer.

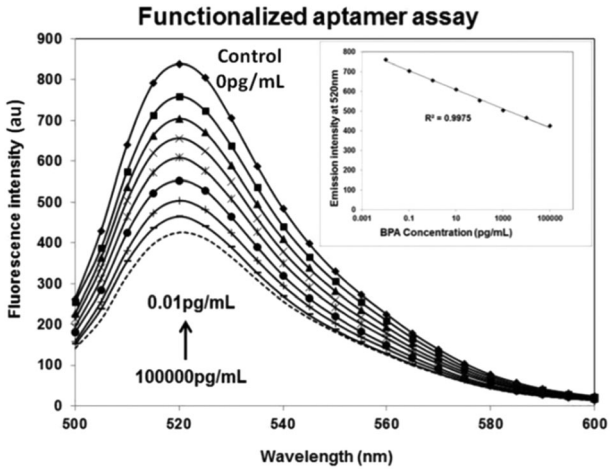

Fig. 4 Fluorescence emission spectra of the functionalized aptamer assay. Inset image shows emission at 520 nm with respect to the BPA concentration.

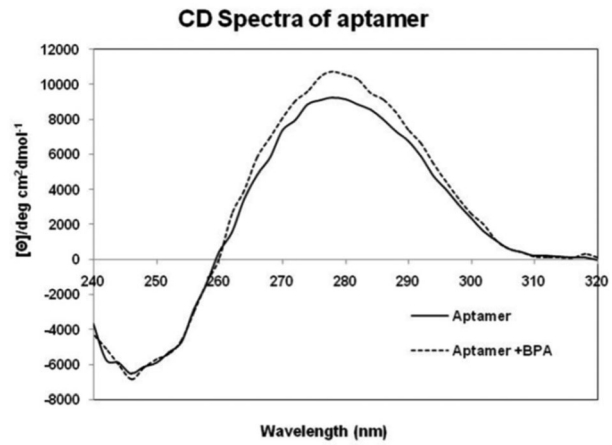

Fig. 5 Circular dichroism spectra showing different spectra for the bound and unbound aptamers.

Hence the aptamers are specific to BPA during analysis without significant cross-reactivity with its analogs. To further confirm the specificity of the aptamer, a scrambled aptamer sequence was selected and functionalized with BHQ and FAM at the 5′and the 3′ end, respectively. Specificity of the scrambled aptamer was studied using CD spectroscopy and fluorimetric assay. To evaluate the performance of the proposed method, we carried out real sample analysis in urine samples (ESI†). The detection limit was found to be 0.1 pg mL^{-1} in urine samples and 0.01 pg mL^{-1} in water samples with the functionalized aptamer.

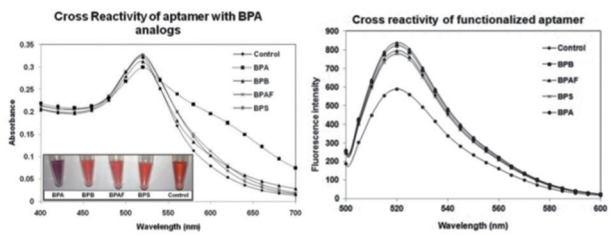

Fig. 6 Cross-reactivity of aptamers with BPA analogues. (a) Aptamer–GNPs assay. (b) Functionalized aptamer assay.

In summary, we proposed and performed a novel assay for detection of BPA using the 'turn-off' biosensor with a functionalized aptamer tagged with FAM and BHQ where the detection is based on the quenching of fluorescence emission upon binding of BPA with the functionalized aptamer. In this study we were able to detect up to 0.01 pg mL^{-1} of BPA, whereas using the aptamer–gold nanoparticle based method the detection limit was 0.1 ng mL^{-1}. Detection of BPA is directly based on the functionalized aptamer alone, whereas in the case of the aptamer–GNPs method several parameters regulate the detection. Hence the proposed method is superior compared to the aptamer–GNPs method in terms of simplicity and the detection limit. Despite the usage of instruments it has many advantages such as a single step process, sensitive, rapid, less expensive and compact, which surmounts other analytical and aptamer based methods. In the case of other matrices *e.g.* in solid samples, a solid phase extraction is necessary in order to estimate BPA. Generally aptamer based methods are very much practical for onsite monitoring of BPA being easy to perform in contrast to conventional analytical methods. Using the principle of the functionalized aptamer sensing method it is possible to detect various contaminants in water like endocrine disrupting compounds, persistent organic pollutants, heavy metals, *etc.*, by having respective aptamers tagged with FAM and BHQ at sensitive concentrations.

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