Electronic Supplementary Information

Boronic acid chemistry for fluorescence-based quantitative DNA sensing

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Materials

The reagents iron(II) sulfate heptahydrate and iron(III) chloride required for the synthesis of magnetite nanoparticles (NPs) were procured from Sigma-Aldrich, whereas ammonia solution required for the same was obtained from SD Fine Chemicals India Ltd. The reagents such as gold(III) chloride trihydrate and tetraoctylammonium bromide required for the synthesis of gold shell over the magnetite nanoparticles were also procured from Sigma-Aldrich. Besides, the fluorescent molecule 3-dansylaminophenylboronic acid was also sourced from Sigma-Aldrich, while the solvents and reagents such as methanol, toluene, dimethyl sulfoxide (DMSO), Tween 20, ethidium bromide (EtBr) and 1,4-dithiothreitol (DTT) were procured from Merck. All the oligonucleotides used in this study were purchased from Eurofins India Pvt. Ltd. A 6X loading buffer was procured from Takara for the gel electrophoresis study.

Table S1. The sequences of the oligos used in the study.

Sample	Sequence 5'-3'	GC content / Melting temp (°C)
Thiolated probe	SH-AAAAAAAAAAGCTGCACGACACTCATACT	34% / 55.9
cDNA	GCCATGGCGTTAGTATGAGTGTCGTGCAGC	57% / 65.7
cRNA	GCCAUGGCGUUAGUAUGAGUGUCGUGCAGC	57% / 65.7
ncDNA	GCGGAAGCTTCTACTTTTTCTGCATCAAGC	47% / 66.8

Synthesis of Fe_3O_4@Au core-shell nanostructures

Fe_3O_4 NPs were synthesized through the coprecipitation method as per the literature protocol.[1] Briefly, 50 mL of water containing 0.5 M Fe^{2+} and 1.0 M Fe^{3+} salts were heated to 80 °C. to which about 35 mL of 25% ammonia solution was added and stirred for 30 min. The obtained magnetite nanoparticles were washed thrice with water, twice with methanol, recovered and dried at 60 °C in a hot air oven.

Fe_3O_4@Au core-shell nanostructures were synthesized using a recently developed solid-state synthesis route.[2] For this, gold-tetraoctylammonium bromide (Au-TOAB) precursor was synthesized by reacting $HAuCl_4.3H_2O$ aqueous solution with tetraoctylammonium bromide, followed by phase transfer to toluene and drying the organic solvent. About 100 mg of Fe_3O_4 was mixed with 20 mg of Au-TOAB in a clean mortar and pestle and grounded well for 10 min. The contents were then transferred to a Teflon-lined autoclave and calcined at 270 °C for 2 h. The obtained core-shell nanostructures were gently grounded and used for further studies.

Immobilization of thiolated probe over the core-shell nanostructures

The disulfide bond of the thiolated probe was reduced using DTT (in 100 mM sodium phosphate buffer, pH 8.3–8.5) at a 1:5 ratio and incubated at room temperature for 1 hour. After the treatment, the probe was precipitated using ethanol and column purified using DNA binding silica columns and eluted in sterile water. For

immobilization, 100 µL of 20 µM of DTT treated SH-Probe containing 18.82 µg DNA (188.2 ng/µL) was incubated with 50 mg of core-shell nanostructures at 37 °C for 2 h with continuous agitation in 20 mM citric acid (pH: 3.0). The citrate buffer was prepared by dissolving 25.08 mg of trisodium citrate in 5 mL of water and the final pH was adjusted to 3.0 using dilute HCl. After 2 h, the unbound probe was removed and further washed with 100 µL of 20 mM citric acid to wash away any loosely bound/adsorbed probe DNA over the gold surface. The collected fractions were collated and quantified using NanoDrop spectrophotometer. By subtracting the amount of unbound probe from the initial loading, the amount of probe bound to 50 mg of Fe_3O_4@Au nanostructures was estimated to be 5.32 µg, which is equivalent to 106 ng/mg of the solid support. The core-shell nanostructures immobilized probe were dispersed in 500 µL of sterile water and stored at 4 °C till further use.

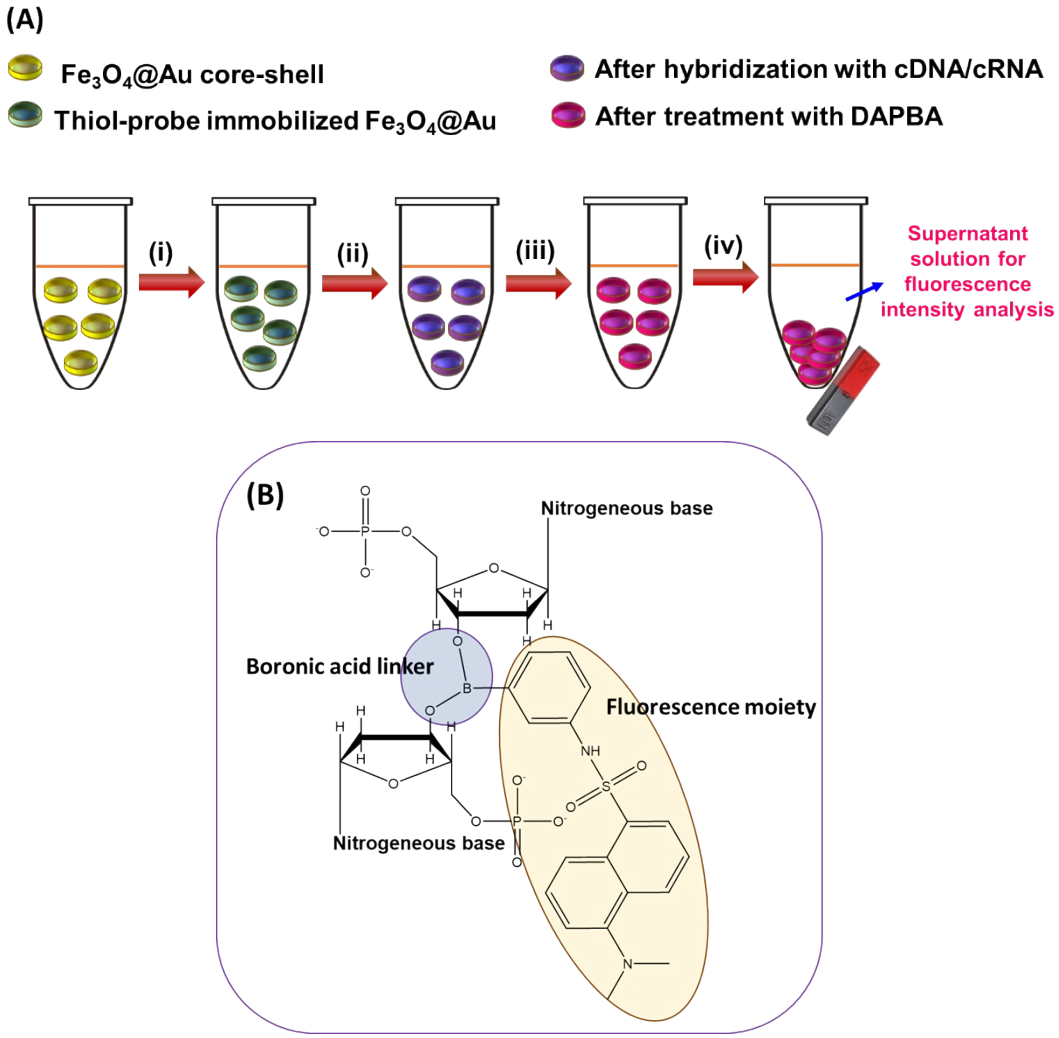
Oligonucleotide sensing

The probe-immobilized core-shell nanocomposite (100 nM w.r.t. the probe) was hybridized with single-stranded cDNA, cRNA or ncDNA. The concentration of the target and non-target oligos were varied from 5 nM to 100 nM in 100 µL reaction volume in water or synthetic serum. The synthetic serum was prepared using 2.2 g/L $NaHCO_3$, 6.8 g/L NaCl, 0.2 g/L $CaCl_2$, 0.1 g/L $MgSO_4$, 0.4 g/L KCl, 0.126 g/L Na_2HPO_4 and 0.026 g/L NaH_2PO_4 at pH: 7.4.The hybridization was carried out by subjecting the mixture to a flash heating at 80 °C for 1 min, followed by 37 °C for 2 h.[3] The flash heating step was introduced to remove any secondary structures in the DNA or RNA strands and thereby enhance the hybridization efficacy. The core-shell nanostructures from the hybridization mixture were magnetically separated to remove any unbound oligos and resuspended in a 100 µL of 1% DMSO aqueous solution containing 100 nM DAPBA. The mixture was incubated further at 37 °C for 30 min, following which the magnetic nanoparticles were once again magnetically removed. Additionally, the mixture was subjected to centrifugation at 12,000 rpm for 1 min to remove any suspended particulates and the fluorescence intensity of the supernatant solution was measured using a plate reader (SpectraMax® iD3, Molecular Devices, USA) at an excitation wavelength of 340 nm and monitoring the emission at 530 nm. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the formula given below.

$$LOD = \frac{3.3 \times standard\ deviation\ of\ the\ intercept}{slope}; LOQ = \frac{10 \times standard\ deviation\ of\ the\ intercept}{slope}$$

Agarose gel Electrophoresis

About 20 µL of the hybridized solution was mixed with 4 µL of 6X loading buffer. The obtained solution mixture was loaded onto a 3 % agarose gel and electrophoresed at 30 V for 3 h in 0.5X TBE buffer (540 mg of Tris base, 275 mg of Boric acid and 30 mg of EDTA; pH: 8.3). Following the gel run, it was stained using 0.5 µg/mL of EtBr solution for 1 h with continuous rocking. After the staining, the EtBr solution was discarded and the gels were washed thrice with sterile distilled water for 10 min and visualized under a UV trans-illuminator.



Scheme S1. (A) Schematic representation of the boronic acid-mediated DNA sensing strategy. (i) treatment of Fe_3O_4@Au with thiolated probe, (ii) hybridization of the immobilized probe with cDNA/cRNA, (iii) treatment of the hybrid with DAPBA and (iv) removal of the core-shell nanostructures to obtain the supernatant solution for the fluorescence intensity measurements. **(B)** The structural depiction of the boronic acid-mediated linking between two DNA strands via the 3‘ termini.

Experimental results

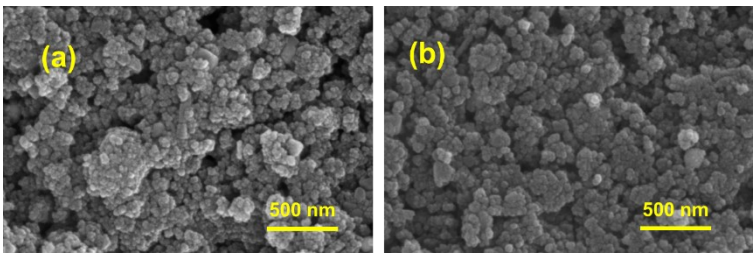


Fig. S1. Field emission scanning electron microscopy images of Fe_3O_4@Au core-shell nanostructures before and after immobilization of the thiolated probe. The surface morphology in both of these cases appear to be identical.

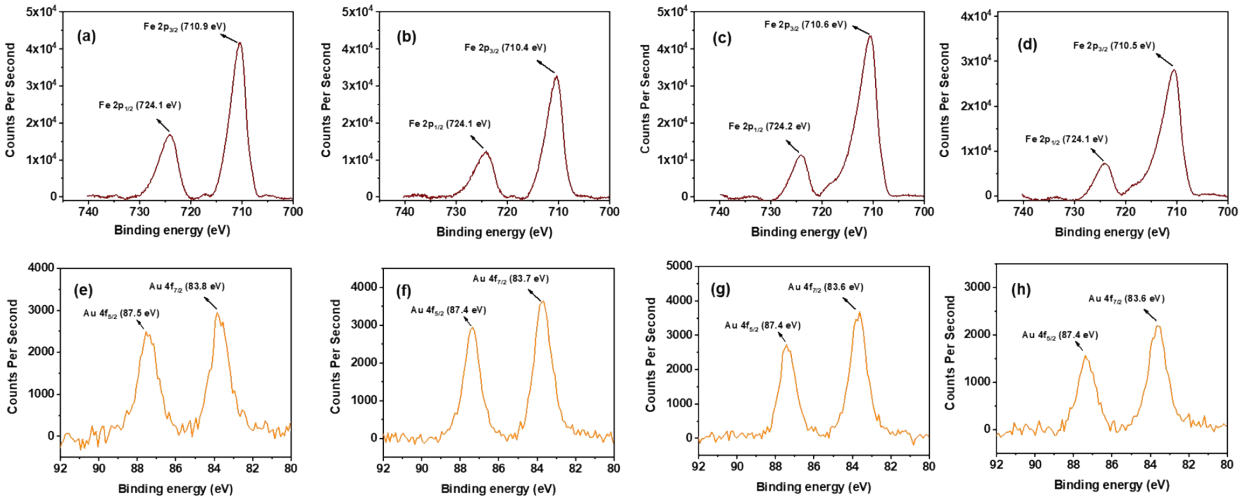


Fig. S2. Fe *2p* and Au *4f* narrow scan spectra of Fe_3O_4@Au core-shell nanostructures before and after probe immobilization: (a, e) before probe immobilization, (b, f) after immobilization of thiolated probe, (c, g) after hybridization with c-DNA and (d, h) after hybridization with c-RNA.

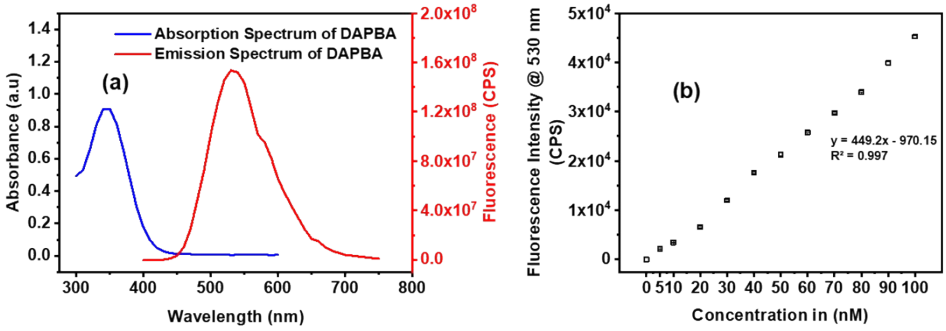


Fig. S3. (a) Absorption and emission spectra of 1 mM DAPBA and (b) standard curve of DAPBA in aqueous solution containing 1% DMSO. Based on the absorption curve, 340 nm wavelength was used for the excitation and 530 nm was used for monitoring the emission.

We additionally performed these sensing experiments in the synthetic serum to explore the applicability of the sensing strategy in clinical serum samples (Fig. 2d). The results revealed a clear reproducibility of the data in comparison to the hybridization mixture that is devoid of electrolytes. Studies with varying concentration of DAPBA on fixed concentrations of the oligos revealed its optimal concentration as 100 nM (Fig. S4). Desorption of the bound DAPBA was studied at pH 3.0, which showed the reversibility of the boronate ester (Fig. S5).

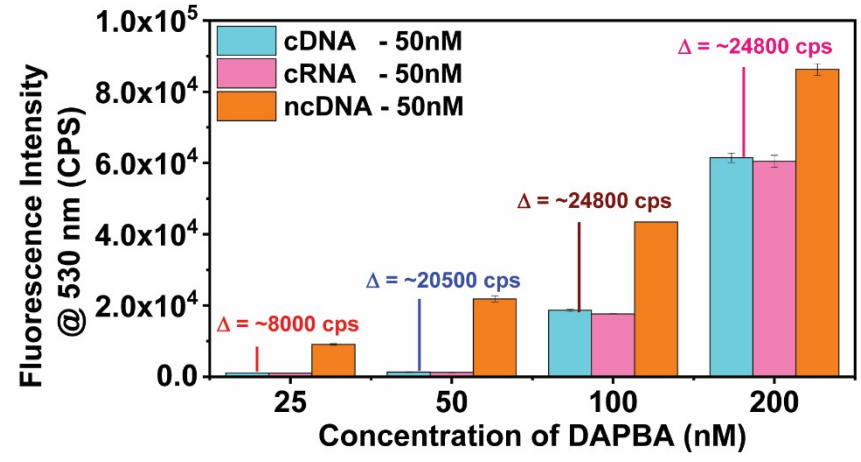


Fig. S4. Fluorescence intensity profiles in the sensing study performed by varying the DAPBA concentration from 25 nM to 200 nM while maintaining the concentration of cDNA, cRNA and ncDNA at 50 nM. The difference in the intensities between the cDNA and ncDNA samples at a given DAPBA concentration is highlighted with a line profile.

The above figure shows the DAPBA binding over the hybridized oligonucleotides as a function of its varying concentration from 25 nM to 200 nM while maintaining a fixed concentration of the oligonucleotides. It can be seen from the figure that the optimal concentration of required DAPBA is slightly higher than that of cDNA (50 nM in the current case). Furthermore, at a fixed concentration of the oligonucleotide, the increase in DAPBA concentration above the optimal level does not affect the differential cps values and thus validating the reliability of the assay.

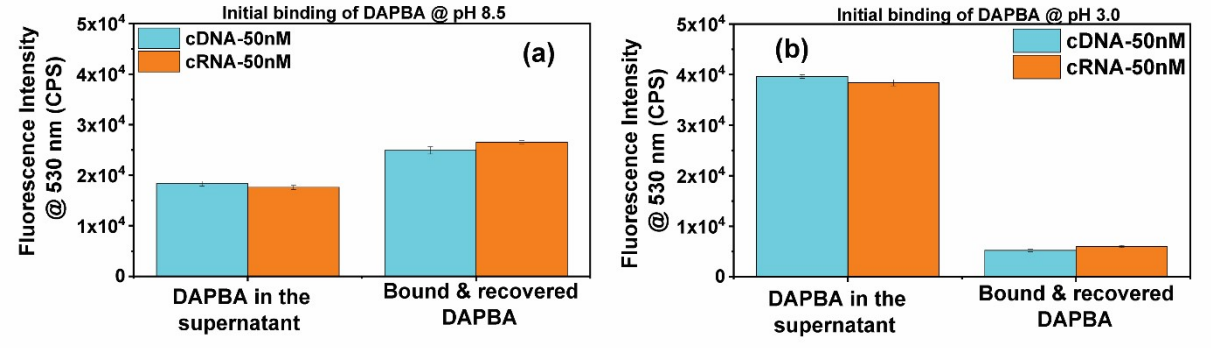


Fig. S5. Reversibility studies of DAPBA at pH 3.0 (using citrate buffer): **(a)** Initial DAPBA binding with dsDNA immobilized over the core-shell nanostructures at pH 8.5, followed by desorption at pH 3.0 and **(b)** initial DAPBA binding with dsDNA immobilized over the core-shell nanostructures at pH 3.0, followed by desorption at pH 3.0. In both the cases, the final pH in the fluorescence intensity measurements was adjusted to 8.5.

Table S2. Comparison of the LOD from a few representative literatures on the fluorescence-based DNA sensing.

Method	**Linear range**	**LOD**	***In situ* amplification**	**Reference**
Activatable Ag nanoclusters beacon for DNA detection	10 – 1000 nM	2 nM	No	Liu et. al.,[4]
DNA-templated Ag nanoclusters	0 – 200 nM	25 nM	No	Y. Zhang et. al.,[5]
Dual-probe fluorescent biosensor	5 pM – 5 nM	3.2 pM	Yes	Y. Zhang et. al.,[6]
Fluorometric detection of multiple oligonucleotides by using RNA-cleaving DNAzymes	1 nM – 400 nM	70 pM	No	Xiang et. al.,[7]
Dual oligosensing using magnetite nanoparticles	10 nM – 100 nM	10 nM	No	Maroju et. al.,[8]
Amyloid β oligomer assay based on abasic site-containing molecular beacon	0 – 70 nM	3.57 nM	No	Zhu et. al.,[9]
A graphene oxide-based fluorescent platform for amyloid-β oligomers	10 nM – 2 mM	1 nM	No	Lit et. al.,[10]
Boronic acid-mediated fluorescence sensing	5 nM – 50 nM	3.5 nM	No	This work

Author Contributions: Pranay Amruth Maroju: Investigation, Formal analysis, Validation. **Ramakrishnan Ganesan**: Conceptualization, Methodology, Resources, Supervision, Writing - review and editing. **Jayati Ray Dutta**: Conceptualization, Methodology, Resources, Supervision, Writing - review and editing.

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