Electronic Supplementary Information

Direct experimental evidence for the boronic acid-mediated bridging of DNA hybrids

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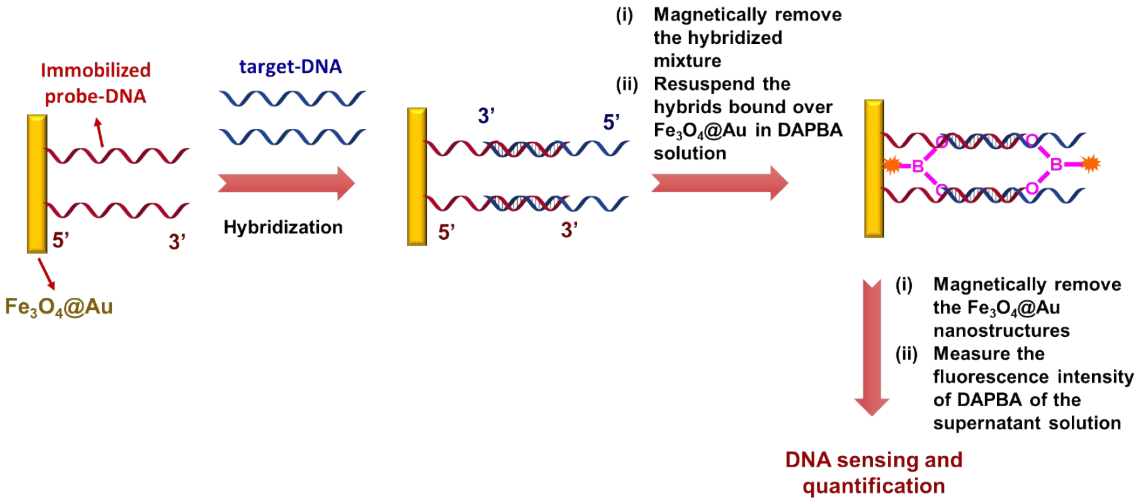
Experimental section

Materials

Iron(II) sulfate heptahydrate and iron(III) chloride for magnetite synthesis were purchased from Sigma-Aldrich. Ammonia solution (25% v/v) used in magnetite synthesis was obtained from Merck, Millipore. The reagents for the synthesis of gold shell such as gold(III) chloride trihydrate ($HAuCl_4.3H_2O$) and tetraoctylammonium bromide (ToABr), and the boronic acid fluorophore, 3-dansylaminophenylboronic acid (DAPBA), were purchased from Sigma-Aldrich. Methanol, toluene, dimethyl sulfoxide (DMSO), Tween 20, ethidium bromide (EtBr), and 1,4-dithiothreitol (DTT) were procured from Merck, Millipore. All the oligonucleotides used in this study were purchased from Eurofins India Pvt. Ltd. The enzyme, terminal deoxynucleotidyl transferase (TdT), used for 3'-end labelling of oligos was procured from Thermo Fischer Scientific and its substrate dideoxynucleoside triphosphate set (ddNTPs) was purchased from Sigma-Aldrich.



Scheme S1. Schematic representation of various steps in the experimental design for boronic-acid mediated quantitative DNA sensing.

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

Sample	Sequence (5'-3')	GC content / Melting temp (°C)
Probe-20(20)	SH-AAAAAAAAAAAAAAAAAAAACCATGAGGTCGGCGAATCCG	32.5/66.38
Probe-20(30)	SH-AAAAAAAAAAAAAAAAAAAAGGGATGTACCCCATGAGGTCGGCGAATCCG	38/71.88
Probe-20(40)	SHAAAAAAAAAAAAAAAAAAAAGCCGACGAGCGGGATGTACCCCATGAGGTCGGCGAATCCG	45/76.92
Probe-10(40)	SH-AAAAAAAAAAGCCGACGAGCGGGATGTACCCCATGAGGTCGGCGAATCCG	54/75
Target-20	CGGATTCGCCGACCTCATGG	65/63.45
Target-30	CGGATTCGCCGACCTCATGGGGTACATCCC	63.33/73.6
Target-40	CGGATTCGCCGACCTCATGGGGTACATCCCGCTCGTCGGC	67.5/80.7
Control-20	TTACCGATAATCCTCCGGGG	55/59.5

Control-30	TTACCGATAATCCTCCGGGGCATAACGAAT	46.67/66.77
Control-40	TTACCGATAATCCTCCGGGGCATAACGAATGCTTATAGGA	45/71.5

Synthesis of Fe_3O_4@Au core-shell synthesis and sensing

Fe_3O_4@Au core-shell nanostructures and the oligonucleotide sensing were performed following our earlier literature reports.[1,2]

End labelling of Probes, Targets/Controls:

The sulfhydryl groups of the thiolated probes were first exposed by cleaving a disulphide bond using DTT (100 mM) in sodium phosphate buffer at pH 8.3– 8.5. Specifically, 10 µg of each thiolated probe was added to a sterile centrifuge tube containing 125 µL of DTT solution and incubated at room temperature for 1 h. The resulting mixture was then subjected to ethanol precipitation to separate the probes from the by-products, followed by purification using DNA-binding silica columns. The purified probes were finally eluted in sterile water for immobilization. To block the 3'-OH groups from oligos, (TdT), a template-independent DNA polymerase that catalyzes the addition of dideoxyribonucleotides to the 3'-OH of single or double-stranded DNA was used. For this, about 10 pmol of each of the single-stranded DNA (probe, target or control) was taken in a sterile PCR tube and mixed with 11 pmol of ddATP in a total volume of 50 µL containing 1x concentration of TdT reaction buffer. A total of 40U (2 µL) of TdT enzyme was then added to each reaction and incubated at 37 °C for 30 minutes in a thermal cycler. After incubation, the enzyme was denatured by heating at 80 °C for 10 min. The obtained end-labelled ssDNA with no 3'-OH groups were subsequently used for immobilization onto Fe_3O_4@Au nanostructures and further hybridization experiments with targets/controls.

Immobilization of the TdT-treated thiolated probes

For immobilization, 100 µL of 5 µM TdT-treated and 10 µM of untreated thiolated-Probes, were 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). After this, the magnetite nanostructures were washed with 100 µL of 20 mM citric acid to remove any loosely bound probe DNA. The unbound DNA in the collected supernatants was quantified using agarose gel and thus ascertained the successful immobilization of the probes. The probe-immobilized core-shell nanostructures were dispersed in 500 µL of sterile water and stored at 4 °C till further use.

Isothermal titration calorimetry (ITC):

The ITC experiments were performed with Microcal PEAQ-ITC (Malvern, Germany). About 0.2 mL of dsDNA (probe-10(40)) was injected into a coin-shaped Hastelloy cell using a Hamilton syringe. The ligand, DAPBA, was added sequentially in 2 µL aliquots for 18 injections with 15 s duration and constant stirring at 750 rpm, at intervals of 150 s at 25 ºC. The differential heat produced during each injection was plotted against time. It can be noted that all the DNA solutions contained 1% DMSO to match the solvent composition of DAPBA.

Experimental results

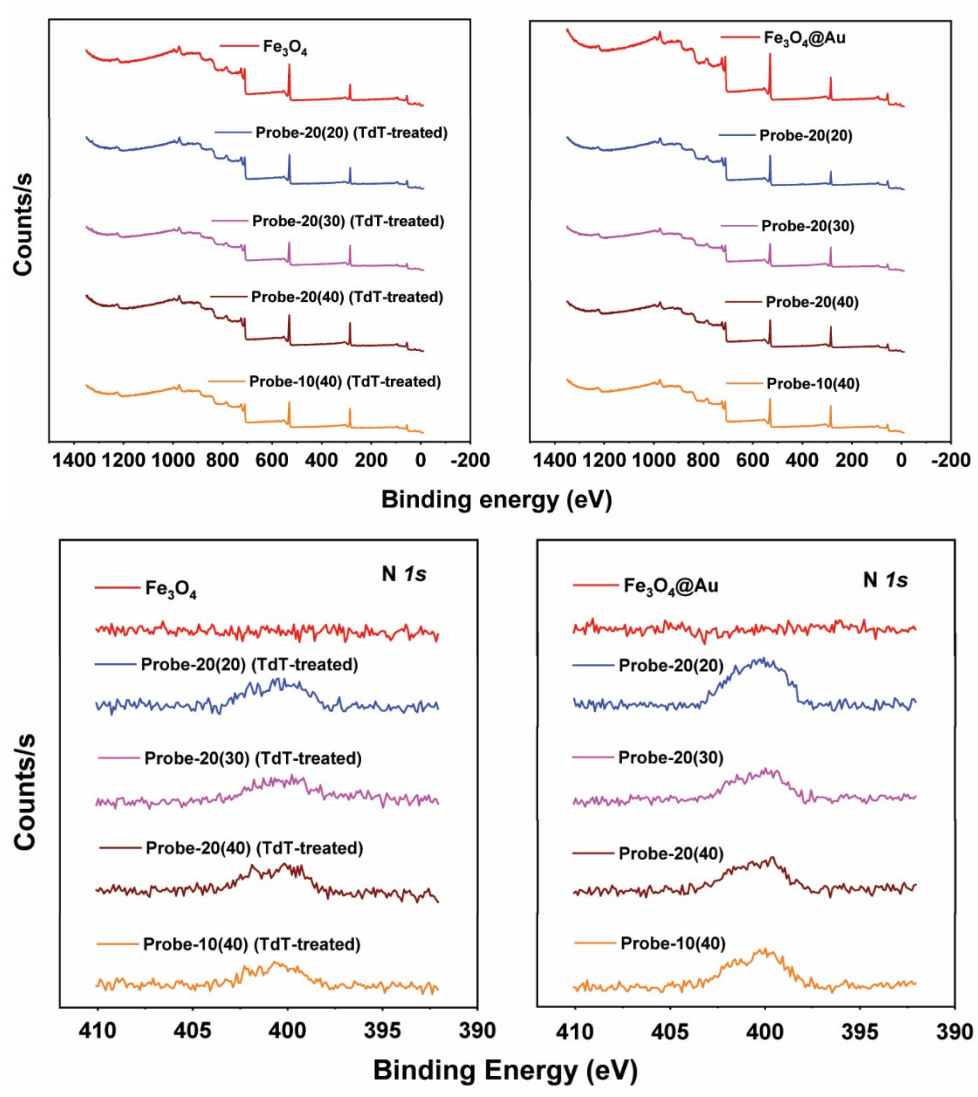
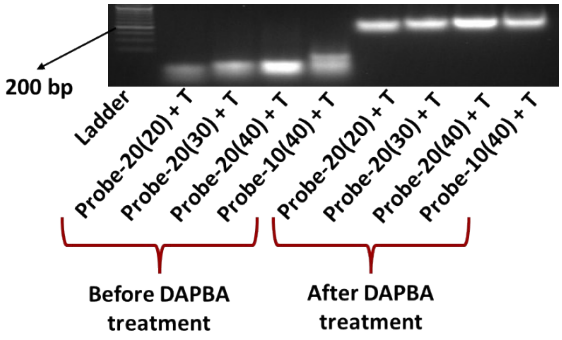


Fig. S1. Survey scan (top panel) and N *1s* narrow scan XPS spectra of pristine Fe_3O_4, Fe_3O_4@Au, Fe_3O_4@Au immobilized with TdT-treated probes and Fe_3O_4@Au immobilized with untreated probes.

Table S1. Comparison of the dissociation constant (K_D) of DNA with different ligands:

Interaction	K_D Values (M)	Reference
DNA with EtBr (intercalating agent)	$0.31\text{-}5.2 \times 10^{-6}$	3,4
DNA with Polymerase (Enzymes)	$0.8\text{-}100 \times 10^{-9}$	5
Chromatin with Enzyme Inhibitors (Proteins)	$0.38\text{-}16 \times 10^{-6}$	6
DNA with Propidium Iodide (dye)	1.8×10^{-6}	4
DNA with Elasmin (anti-tumour drug)	2.8×10^{-6}	7
DNA with DAPBA (Fluorescent molecule)	$\sim 1\text{–}35 \times 10^{-6}$	This study

The table reveals that the binding strength of DNA with boronic acid is similar to the tune of its binding with intercalating agents and drugs.



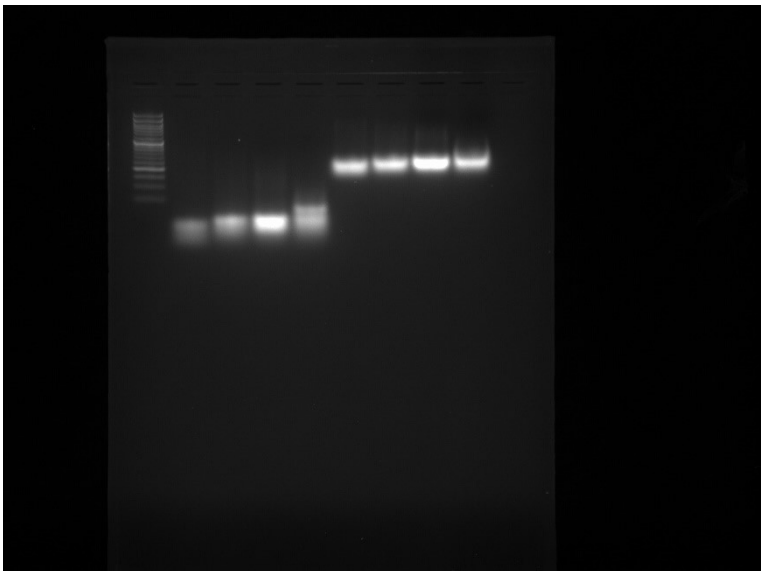
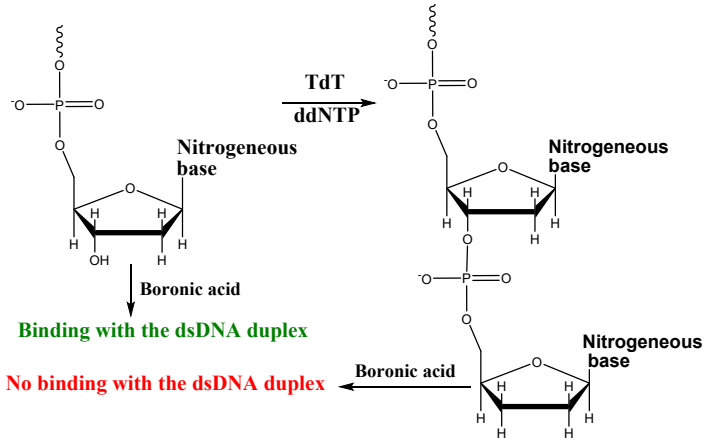


Fig. S2. Agarose gel electrophoresis of DNA hybrids before and after treatment with DAPBA, wherein a 100 μL of 100 nM DAPBA solution was treated with 2 μg of each DNA hybrid such that the concentrations of probe-20(20), probe-20(30), probe-20(40) and probe-10(40) hybridized with their respective targets were ~108, 81, 65 and 72 nM, respectively. The uncropped and unprocessed gel image is provided below the cropped version, wherein the labels remain unchanged.



Scheme S2. The chemical reaction depicting the blocking of 3' hydroxyl moieties of DNA using TdT and ddATP to verify the non-binding event with the boronic acid.

Author Contributions: Pranay Amruth Maroju: Investigation, Formal analysis, Validation. **Anindita Thakur:** 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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