Impedance-based DNA switches for Solving the Boolean Logic Circuit

*Tejal Dube, Deepak Sharma[#], Shalini Gupta and Manojkumar Ramteke**

Supplementary Information

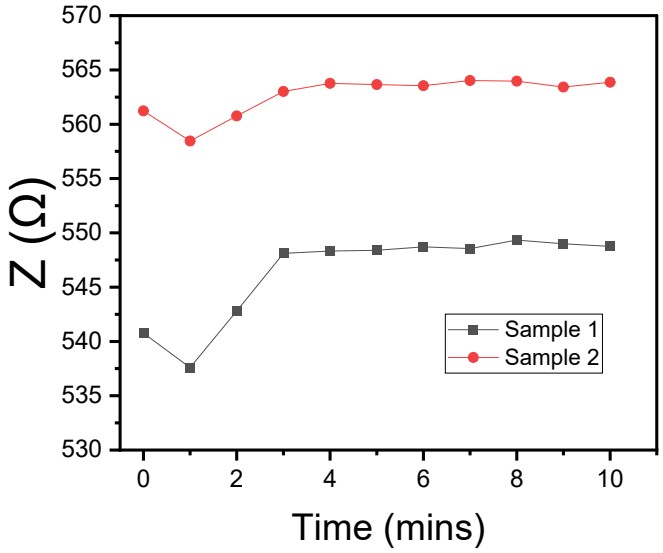

Fig. S1. Time-resolved impedance signals for two independent 100m-oligo samples, showing signal stabilization time of three minutes post sample injection on the electrodes.

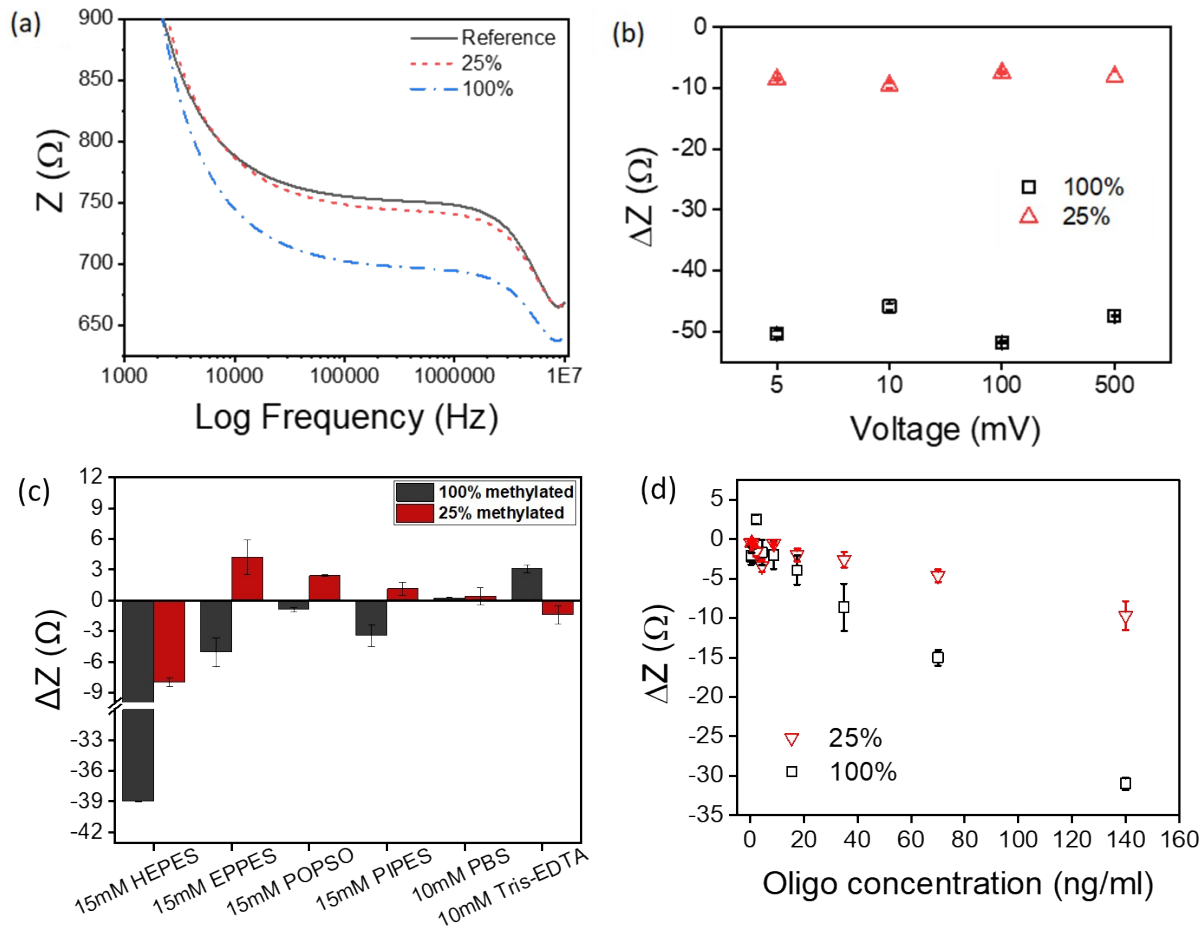

Fig. S2. Effect of different parameters on the impedance response of the system: **(a)** applied frequency, **(b)** applied voltage, **(c)** buffer type, and **(d)** oligonucleotide concentration used. All the data are reported as the mean of triplicate readings +/- 2 SD.

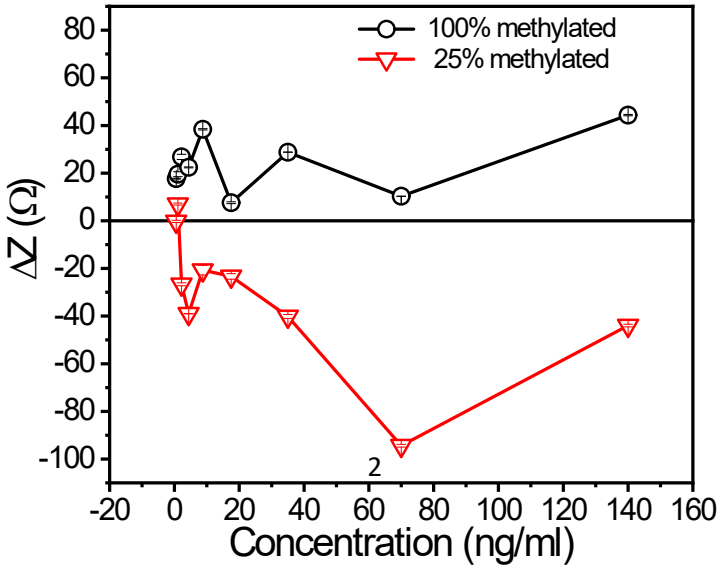

Fig. S3. Interference study with healthy human blood plasma samples spiked with 25m- or 100m-oligos. To obtain the samples, 1 µl of plasma was mixed with 19 µl of the prepared methyl oligo solution (98 µl of 15 mM HEPES buffer + 1 µl of 7 µg/ml DNA oligomer + 1 µl EB). Results are reported as the mean of duplicate readings from independent test samples +/- 2 SD.

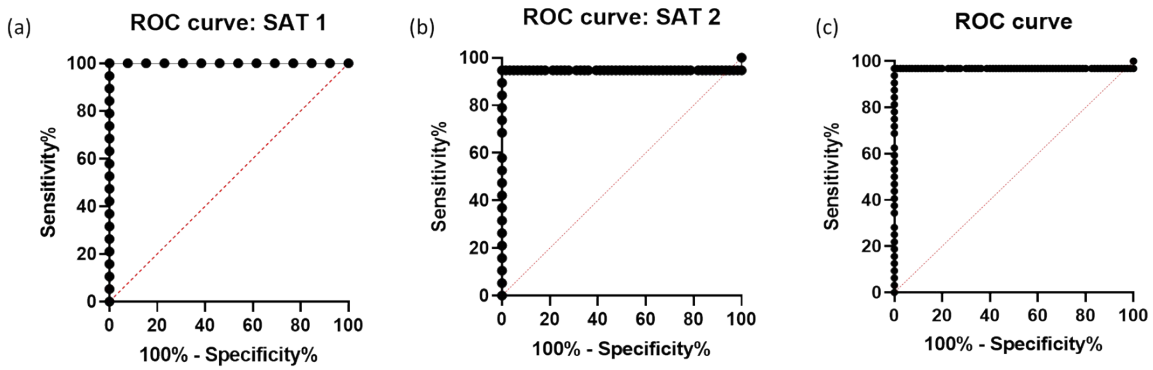

Fig. S4. Receiver Operating Characteristic (ROC) and Area Under Curve (AUC) for **(a)** SAT 1 problem, **(b)** SAT 2 problem, and **(c)** Overall sensitivity and specificity analysis of the system.

Methodology:

Calibration of the system: A single set of electrodes was used for all the measurements. The electrode surface was revived by applying HEPES buffer for 2-3 times until the reference buffer reading was achieved. If not then 1M NaOH was used to remove bound DNA on the electrode surface. For changing buffer solution especially in studying the effect of various buffer solutions on the system, 20 ul volume of respective buffer was used as reference solution and the buffer with tris (in which DNA was suspended) was used as control, to calibrate the

electrode after every reading. Similarly, in the case of varying segments of DNA, a constant reference buffer HEPES was used at least 3-5 times to validate the known signal provided by the HEPES buffer. This eliminates the possibility of having zero error caused by the bound DNA from the previous sample solution.

Formation of NAND gate. STEP 1: 15 mM HEPES buffer (98 µl) to which 7 µg/ml DNA oligomers (1 µl) and 110 mM NaCl (1 µl) were added. Followed by recording the impedance signals. STEP 2: The DNA sample solution was mixed with 2 µl of magnetic beads followed by 5-10 mins of vortexing at medium speed. The solution was then placed in a magnetic stand for another 1 min to separate the magnetic beads bound with the oligomers. The supernatant was replaced with EB while keeping the tube on the magnetic stand, and the mixture was vortexed at medium speed for 4-5 min to obtain purified oligo samples. Next, the concentration of extracted DNA is recorded using Nanodrop. STEP 3: 7 µg /ml DNA oligomers (1 µl) and EB (1 µl) were added to 15 mM HEPES buffer (98 µl). The samples were either heated at 95 ^{O}C for 5 min followed by slow cooling at room temperature to reduce the impedance values or introduced to 15 mM HEPES buffer of pH 6.9 instead of pH 7.4 followed by recording the impedance signals.

Formation of NOR gate: STEP 1: 15 mM HEPES buffer (98 µl) to which 7 µg /ml DNA oligomers (1 µl) and EB (1 µl) were added. The mixture was heated at 95 ^{O}C for 5 mins followed by slow cooling at room temperature. Followed by recording the impedance signals. STEP 2: 100 µl sample was mixed with 2 µl of magnetic beads followed by 5-10 mins of vortexing at medium speed. The solution was then placed in a magnetic stand for another 1 min to separate the magnetic beads bound with the oligomers. The supernatant was replaced with EB while keeping the tube on the magnetic stand, and the mixture was vortexed at medium speed for 4-5 min to obtain purified oligo samples. Next, the concentration of extracted DNA is recorded using Nanodrop. STEP 3: 7 µg /ml DNA oligomers (1 µl) and EB (1 µl) were added to 15 mM HEPES buffer (98 µl). The samples were either heated at 95 ^{O}C for 5 min followed by slow cooling at room temperature to reduce the impedance values or introduced to 15 mM HEPES buffer of pH 6.9 instead of pH 7.4 followed by recording the impedance signals.