Electronic Supporting Information

Highly efficient Baby Spinach-based minimal modified sensor (BSMS) for nucleic acid analysis

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Materials and methods.

Oligonucleotides (Table S1) were either purchased from Integrated DNA Technologies (IDT) or transcribed in vitro. Autoclaved DEPC (Diethyl pyrocarbonate) treated water was used for

dissolution of oligonucleotides, preparation of buffers and all the assays. Concentrations of oligonucleotides were determined using Nanodrop One^{C} (Thermo Scientific). dNTPs (DNTP100), Taq DNA Polymerase (D4545) and DFHBI (SML1627) were purchased from Sigma. MinElute PCR Purification Kit was purchased from QIAGEN (28006). In vitro transcription was carried out using Ampliscribe T7 High Yield Transcription Kit (AS3107, Epicentre). Tris-HCl (pH 7.40) was prepared from Trizma base (T6066, Sigma) by adjusting pH with conc. HCl. The 5X Sensor buffer (50 mM Tris-HCl (pH=7.4), 500 mM KCl, 50 mM $MgCl_2$) was prepared. All fluorescent spectra were taken using Spectrofluorometer FP-8500 (JASCO). Excitation wavelength was set to 450 nm and emission was taken at 505 nm.

BSMS design

BSMS was designed by fusing the complementary sequence of the target nucleic acid to the 3' end of the Baby Spinach aptamer. Design and sequence of the BSMS is shown in Fig. S1. Secondary structure prediction and a proper folding pattern of all BSMS probes was done before and after binding with the target nucleic acid sequence using NUPACK online program (Fig. S2).[1]

Detailed experimental procedure

The corresponding sensor sequences were ordered as single stranded DNA from IDT and were used as template to create dsDNAs using a forward primer which included a 5' T7 promoter sequence and respective reverse primers. The dsDNAs were transcribed in vitro using Ampliscribe T7 High Yield Transcription Kit.

General procedure for Polymerase chain Reaction. 50 µL PCR reaction mixture contained 0.5 µM of respective forward and reverse primer, 1X PCR Buffer, 2.5 mM $MgCl_2$, 150 µM dNTPs, 0.05 U/µL of Taq DNA polymerase and 10 ng of Baby Spinach template. For scale up, 500 µL of PCR was performed with a 1 min denaturation cycle at 95°C, followed by 10 cycles of 95°C for 1 min., 55°C for 1 min. and 72°C for 1 min with a final extension at 72°C for 10 min respectively. PCR products were visualized on 1.5% agarose gels in TAE buffer followed by purification using the MinElute PCR Purification Kit (QIAGEN). Concentrations of purified dsDNA were determined using Nanodrop One^{c} and respective dsDNA were used as templates for in vitro T7 transcription reactions.

In vitro transcription. RNA was transcribed from dsDNA template by in vitro transcription reactions at 37°C using Ampliscribe T7 High Yield Transcription Kit as per manufacturer's protocol. Transcription products were visualized on 8% Urea PAGE in TBE to ensure that the transcribed RNAs were of the proper length. The transcription products were later purified from gels using crush and soak method. Concentrations of purified sequences were determined using Nanodrop One^{c}.

Optimization of DFHBI Concentration. The 60 µL reaction mixture contained 0.25 µM BSMS and 0.25 µM of miRNA-263a with varied concentrations of DFHBI respectively (0.125 µM, 0.25 µM, 0.5 µM, 1.0 µM, 2.0 µM, 2.5 µM and 4.0 µM) in sensor buffer. The control sample (without analyte) was taken with the highest DFHBI concentration (4 µM). The reaction was incubated at room temperature for 30 min and the fluorescent spectra were recorded. All the experiments were done in triplicate and data of three independent experiments was processed using Microsoft Excel (Fig. S3).

Specificity in presence of yeast tRNA. The 60 µl reaction mixture contained 2.5 µM DFHBI, 0.25 µM BSMS, 0.25 µM of analyte miRNA-263a or miDNA-263a respectively and 1 µl of 10mg/ml yeast tRNA in sensor buffer. Control samples contained 2.5 µM DFHBI, 0.25 µM BSMS, 1 µl of 10 mg/ml yeast tRNA in sensor buffer and were ran parallel. The reaction was incubated at room temperature and fluorescent spectra were measured after 30 min. Data of three independent experiments was processed using Microsoft Excel (Fig. S6).

Table S1: Oligonucleotide sequences used in this study

Purchased from IDT	
Name	**Sequence**
dme-miR-263a RNA	AAUGGCACUGGAAGAAUUCACGGG
dme-miR-263a DNA	AATGGCACTGGAAGAATTCACGGG
dme-263a-5′-extn	*TCTTCATT*AATGGCACTGGAAGAATTCACGGG
dme-263a-3′-extn	AATGGCACTGGAAGAATTCACGGG*CTCGGTAA*
dme-263a-5′-3′-extn	*TCTTCATT*AATGGCACTGGAAGAATTCACGGG*CTCGGTAA*
10 Base target analyte	GTCCCGAGAG
13 Base target analyte	AGCGTCCCGAGAG
16 Base target analyte	CCCAGCGTCCCGAGAG
37 Base target analyte	GCGGCATGGGTATGGGCCACTGACACAACACAAGGAC
dme-263a-1M	AATG**C**CACTGGAAGAATTCACGGG
dme-263a-1M mut10	AATGGCACT**T**GAAGAATTCACGGG
api-263a	AATGGCACTG**A**AAGAATTCACGGG
dme-263a-2M	AATG**C**CACTG**C**AAGAATTCACGGG
dme-263a-3M	AATG**C**CACTG**C**AAGAATTCAC**C**GG
In vitro transcribed sequence	
Baby Spinach	GGUGAAGGACGGGUCCAGUAGUUCGCUACUGUUGAGUAGAGUGUGAGCUCC
BSMS-10	GGUGAAGGACGGGUCCAGUAGUUCGCUACUGUUGAGUAGAGUGUGAGCUCC CTCTCGGGAC
BSMS-13	GGUGAAGGACGGGUCCAGUAGUUCGCUACUGUUGAGUAGAGUGUGAGCUCC CTCTCGGGACGCT
BSMS-16	GGUGAAGGACGGGUCCAGUAGUUCGCUACUGUUGAGUAGAGUGUGAGCUCC CTCTCGGGACGCTGGG
BSMS (24)	GGUGAAGGACGGGUCCAGUAGUUCGCUACUGUUGAGUAGAGUGUGAGCUCC CCCGUGAAUUCUUCCAGUGCCAUU
BSMS-1U	GGUGAAGGACGGGUCCAGUAGUUCGCUACUGUUGAGUAGAGUGUGAGCUCC UCCCGUGAAUUCUUCCAGUGCCAUU
BSMS-2U	GGUGAAGGACGGGUCCAGUAGUUCGCUACUGUUGAGUAGAGUGUGAGCUCC UUCCCGUGAAUUCUUCCAGUGCCAUU
BSMS-4U	GGUGAAGGACGGGUCCAGUAGUUCGCUACUGUUGAGUAGAGUGUGAGCUCC UUUUCCCGUGAAUUCUUCCAGUGCCAUU
BSMS-8U	GGUGAAGGACGGGUCCAGUAGUUCGCUACUGUUGAGUAGAGUGUGAGCUCC UUUUUUUUCCCGUGAAUUCUUCCAGUGCCAUU
BSMS-37	GGUGAAGGACGGGUCCAGUAGUUCGCUACUGUUGAGUAGAGUGUGAGCUCC GUCCUUGUGUUGUGUCAGUGGCCCAUACCCAUGCCGC

Mutated bases are bold and underlined, Recognition sequence in respective sensors is underlined, 5', 3'-extensions of target analyte sequences are in Italic.

Figure S1. Detailed design of BSMS

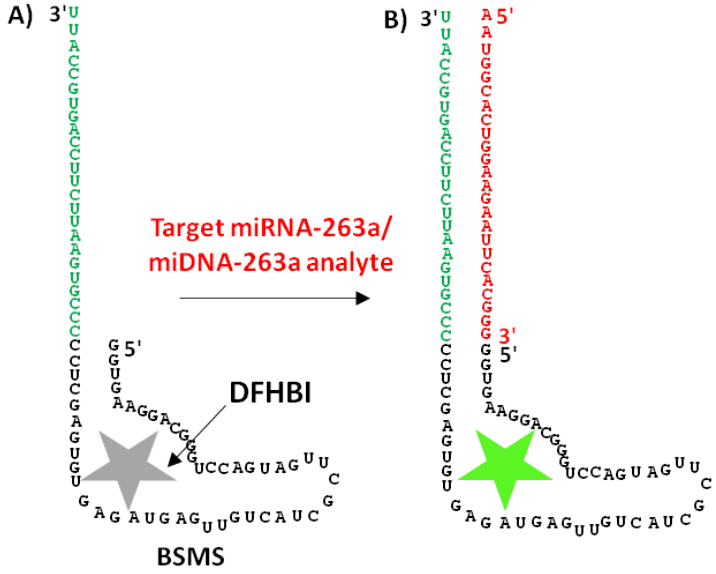

Figure. S1. A) Detailed design of BSMS designed by fusing RNA sequence complementary to dme-miRNA-263a (green) to the 3`end of the Baby Spinach RNA aptamer (black) (B) BSMS in presence of target miRNA-263a (red).

Figure S2. Folding pattern of sensors generated by NUPACK

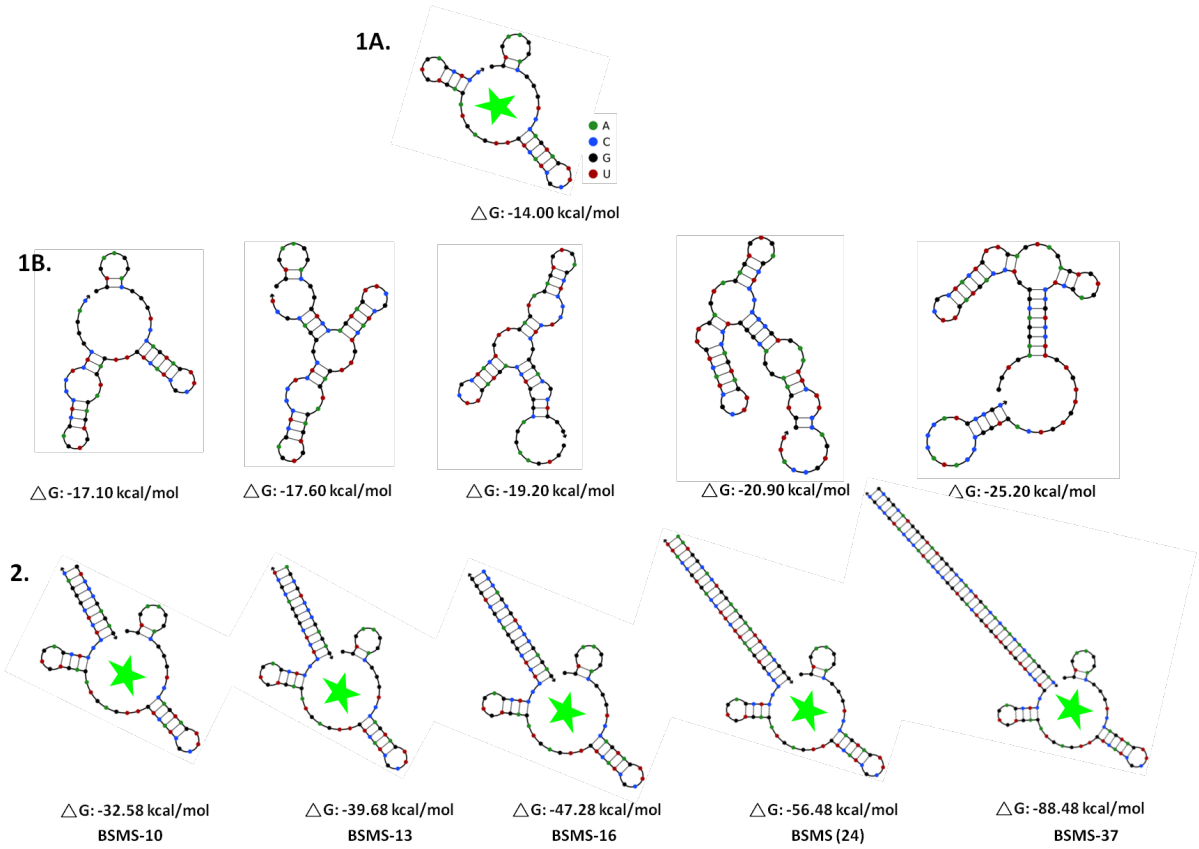

Figure S2. Secondary structure predictions and folding pattern of different BSMS probes before and after binding with the target sequence. 1A) Baby Spinach B) BSMS-10, BSMS-13, BSMS-16, BSMS (24) and BSMS-37 in absence of target nucleic acid sequence using NUPACK 2) BSMS-10, BSMS-13, BSMS-16, BSMS (24) and BSMS-37 in presence of target nucleic acid sequence using NUPACK

Figure S3. Optimization of DFHBI Concentration using BSMS

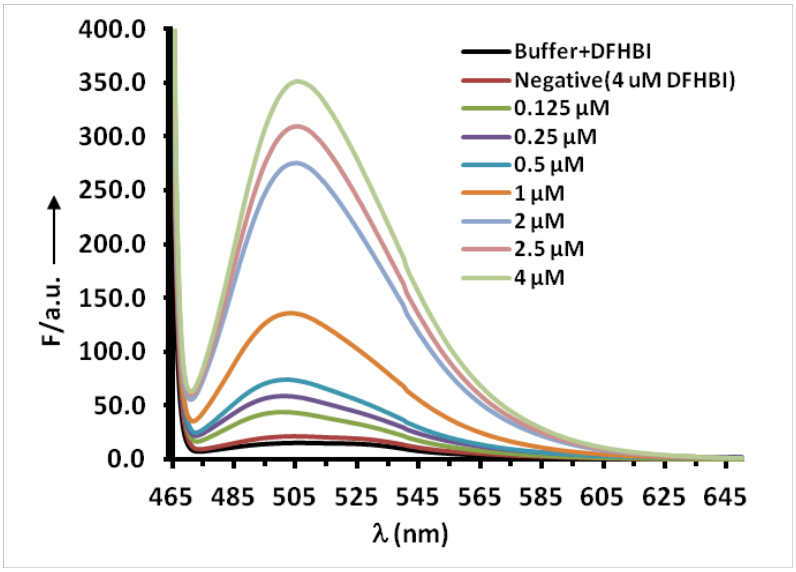

Figure S3. Optimization of DFHBI Concentration. Fluorescent response of BSMS in varied concentrations of DFHBI. Each reaction contained 0.25 µM BSMS, 0.25 µM target miRNA-263a and varied concentrations of DFHBI as mentioned in Figure. Negative was taken with highest concentration of DFHBI (4 µM). The reaction was incubated at room temperature for 30 min.

Figure S4. Fluorescence intensities of BSMS with 1U, 2U, 4U and 8U linker between Baby Spinach and target recognition sequence

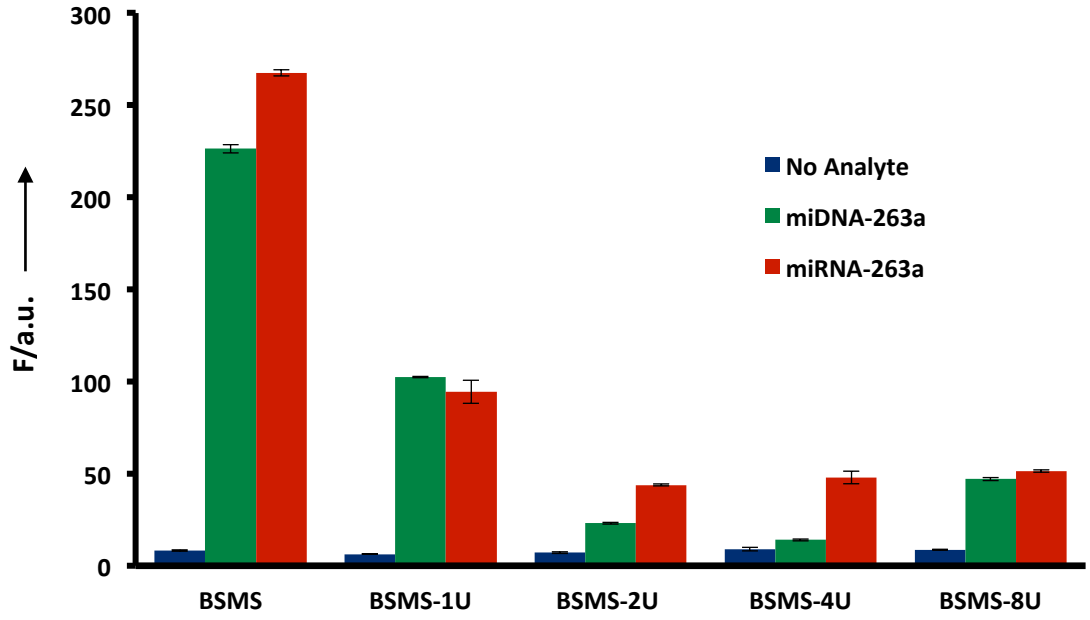

Figure S4. Effect of linker on fluorescence intensities of BSMS. Each 60 µl reaction contained 0.25 µM BSMS or BSMS- 1U/2U/4U/8U, 0.25 µM of analyte miRNA-263a or miDNA-263a, 2.5 µM DFHBI in sensor buffer respectively. The reaction was incubated at room temperature for 30 min.

Figure S5. Fluorescence intensities of BSMS with target sequence extension at ends

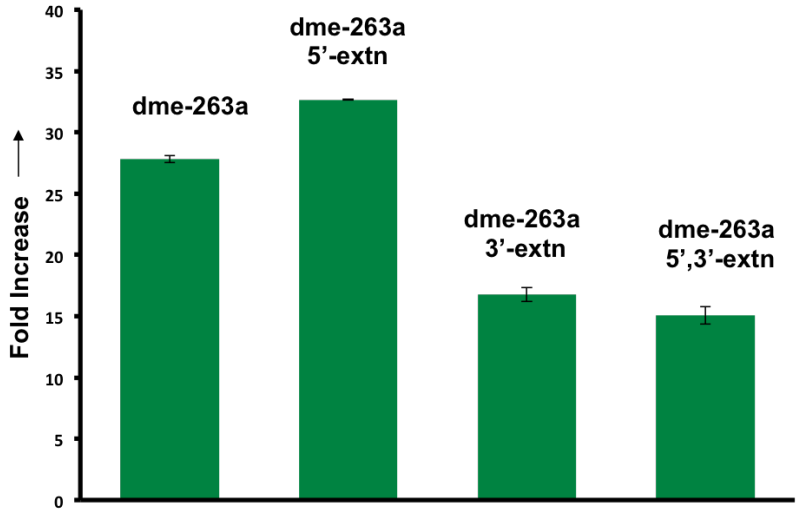

Figure S5. Fluorescence fold change of BSMS compared with 8-nucleotide extensions of the analyte sequence at its 5'end (dme-263a-5'-extn), 3'end (dme-263a-3'-extn) and at both 5'and 3'ends (dme-263a-5'-3'-exten). Each 60 µl reaction contained 0.25 µM BSMS, 0.25 µM of corresponding analyte, 2.5 µM DFHBI in sensor buffer respectively. Fold increase in fluorescence intensities was calculated as: fluorescence intensity of BSMS with analyte divided by fluorescence intensity of BSMS without analyte.

Figure S6. Fluorescence intensities of BSMS in presence of Yeast tRNA

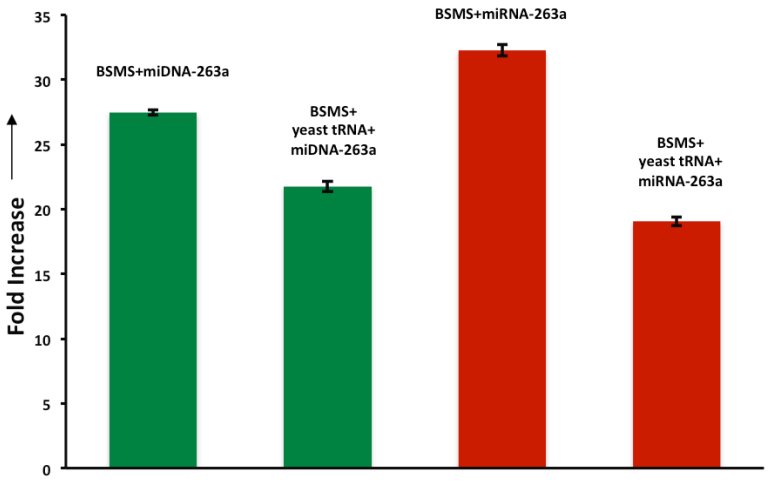

Figure S6. Fluorescence folds Increase of BSMS in presence of Yeast tRNA. Each 60 µl reaction contained 2.5 µM DFHBI, 0.25 µM BSMS, 1 µl of 10 mg/ml yeast tRNA and 0.25 µM of analyte miRNA-263a or miDNA-263a in sensor buffer respectively. The reaction was incubated at room temperature for 30 min. Fold increase in fluorescence intensities was calculated as: fluorescence intensity of BSMS with target analyte divided by fluorescence intensity of BSMS without analyte.

Figure S7. Comparison of Fluorescence intensities of BSMS with different single base mismatch analytes

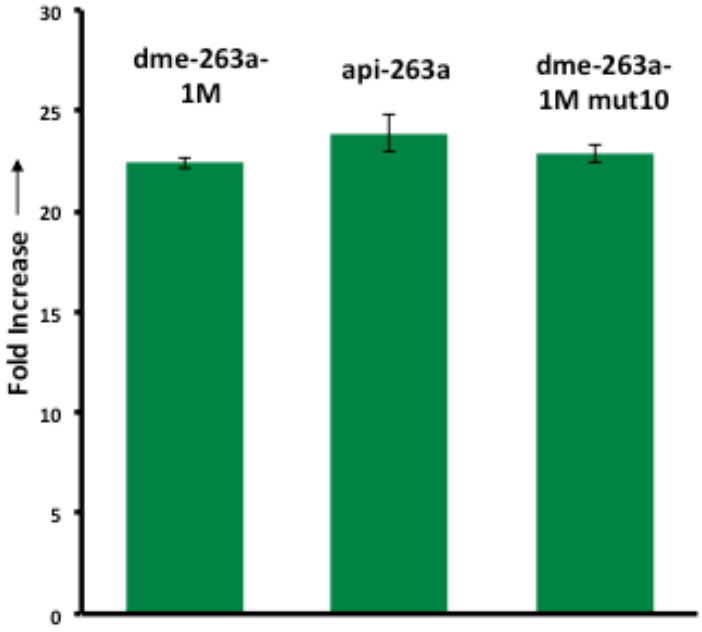

Figure S7. Fluorescence fold change of single base mismatch of different analyte sequence. The results shows only a small difference in fluorescence between different single base mismatch sequences (dme-263a-1M: 22.4 folds, api-263a: 23.8 folds and dme 263a-1M mut10: 22.8 folds). The analyte sequence without any mismtach shows fluorescence fold change of 28 folds (Fig. 4). Each 60 µl reaction contained 2.5 µM DFHBI, 0.25 µM BSMS, 0.25 µM of corresponding single base mismatch sequence in sensor buffer respectively. The reaction was incubated at room temperature for 30 min. Fold increase in fluorescence intensities was calculated as: fluorescence intensity of BSMS with target analyte divided by fluorescence intensity of BSMS without analyte

Table S2: Theoretical estimate of thermodynamic parameters of BSMS with target RNA/DNA analytes

	ΔH kcal/mol	ΔS e. u.	ΔG kcal/mol	T_m °C
dme-miR-263a RNA	-223.0	-589.1	-40.3	85.4
dme-miR-263a DNA	-191.4	-516.6	-37.4	75.1

Thermodynamic parameters were calculated using DINAMelt Web Server of mFold program[2] under two-state-melting tool by keeping Na^+ ion concentration 1 M (default ion concentration for RNA T_m prediction).

References.

[1]http://www.nupack.org/
[2]http://unafold.rna.albany.edu/?q=DINAMelt/Two-state-melting