Supporting Information

Split Dapoxyl Aptamer for Sequence-Selective Analysis of NASBA amplicons

Nanami Kikuchi[a], Adam Reed,[a] Yulia V. Gerasimova,[a*] Dmitry M. Kolpashchikov[a,b*]

a) Chemistry Department, University of Central Florida, 4111 Libra Drive, Orlando, 32816, Florida, USA
b) Burnett School of Biomedical Sciences, University of Central Florida, Orlando, 32816, Florida, USA
E.mail: Dmitry.kolpashchikov@ucf.edu; Yulia.Gerasimova@ucf.edu

Table of content

1. Materials and instruments.

All oligonucleotides were purchased from Integrated DNA Technologies, Inc. (Coralville, IA). DNAse free water was purchased from Fisher Scientific and used for all assays including buffers, and for dissolution of oligonucleotides. Concentrations of oligonucleotide were determined based on UV light absorption at 260 nm. 2-(-4'-Fluorosulfonylbenzoylamino)-4"-dimethylaminoacetophenone (**dapoxyl-SEDA**) and intermediate product dapoxyl fluorine (**dapoxyl-F**) was synthesized as described.[1] KCl and $MgCl_2$ were purchased from Fisher Scientific. Trizma Hydrochloride (Tris-HCl), pH 7.40 was purchased from Sigma Aldrich. Buffer 1 contained 20 mM Tris-HCl, pH 7.4, 200 mM KCl, 10 mM $MgCl_2$. NASBA buffer contained Tris-HCl, pH 8.5, KCl, $MgCl_2$, DTT, Dimethyl Sulfoxide (Life Sciences Advanced Technologies Inc.). Fluorescent spectra were recorded using Fluorescence Spectrometer LS55 (PerkinElmer). Unless otherwise noted, excitation wavelength was set to 390 nm and emission was taken at 505 nm. Time dependence experiments were performed using a Cary Eclipse Fluorescence Spectrophotometer (Agilent). Zika Virus (strain 1840) obtained from World Reference Center for Emerging Viruses and Arboviruses. grown in Vero cells in DMEM media containing 10% fetal bovine serum. Viral RNA was extracted using Trizol LS (Life Technologies) and quantified via absorbance at 260 nm using a NanoDrop.

2. Table SI 1: oligonucleotides used in this study

Name	Sequence	Purification
DAP-10	caattacgggggagggtgtgtggtcttgcttggttcgtattg	SD
SDA_m1	gtgttgtgt tt ct acg gggg a ggg tgtgt gg tctt ggtcat	SD
SDA_f1	atgacc ttggtt cgt ag /teg/ cag tgg ccc ata ccc atg c	SD
A_m	gc ggcatgggtatgggcc actg acacaa c ac aa ggac	SD
A_{mm}	gc ggcatgggtatgggcc actg acataa c ac aa ggac	SD
f	atgacc ttggtt cgt ag cag tgg ccc ata ccc atg c	SD
f-teg	atgacc ttggtt cgt ag /*teg*/ cag tgg ccc ata ccc atg c	SD
f-tt	atgacc ttggtt cgt ag *tt* cag tgg ccc ata ccc atg c	SD
f-teg2x	atgacc ttggtt cgt ag /*teg*/cag tgg ccc ata ccc atg c	SD
m1	gtgttgtgt ctt acg gggg a ggg tgtgt gg tctt ggtcat	SD
m2	tgttgtgt ctt acg gggg a ggg tgtgt gg tctt ggtcat	SD
m3	gttgtgt ctt acg gggg a ggg tgtgt gg tctt ggtcat	SD
m4-tt	gtgttatgt tt ct acg gggg a ggg tgtgt gg tctt ggtcat	SD
m4-teg	gtgttatgt /*teg*/ ct- acg gggg a ggg tgtgt gg tctt ggtcat	SD
m4-teg2x	gtgttgtgt /*heg*/ ct- acg gggg a ggg tgtgt gg tctt ggtcat	SD
m1-teg	gtgttgtgt /*teg*/ ctt acg gggg a ggg tgtgt gg tctt ggtcat	SD

Name	Sequence	Purification
NASBA reverse primer	cac aac gac cgt cag ttg aa	SD
NASBA forward primer	aat tct aat acg act cac tat agg gag aag ggc agt caa gca agc ctg gga aga	SD
Z-147	GGGAGAAGGGCAGUCAAGCAAGCCUGGGAAGAUGGUAUCUGCGGGAUCUCCUCUGUUUCAAGAAUGGAAAACAUCAUGUGGAGAUCAGUAGAAGGGGAGCUCAACGCAAUCCUGGAAGAGAAUGGAGUUCAACUGACGGUCGUUGUG	Expected NASBA product
Z-64	tct gcg gga tct cct ctg ttt caa gaa tgg aaa aca tca tgt gga gat cag tag aag ggg agc t	SD
SDA_f2	atgacc ttggtt cgt ag/*teg*/ tcc aca tga tgt ttt cca ttc ttg	SD
SDA_m2	agc tcc cct tct act gat c *tt* ct acg gggg a ggg tgtgt ggtctt ggtcat	SD

/teg/, triethylene glycol linkers; /*heg/*, hexoethylene glycol linkers; SD, standard desalting; SNV sites are underlined; RNA sequences are in upper case; linkers between analyte binding arms and the aptameric portion of SSA strands are shown in cyan.

3. Detailed Experimental Procedure

General Fluorescent assay for DAP-10. **Dapoxyl-F** (0.5 μM) and **DAP-10** (0.58 μM) were added to 30 μL of Buffer 1 (20 mM Tris-Cl, pH 7.4, 200 mM KCl, 10 mM $MgCl_2$). Total volume was adjusted to 60 μL by water. Control samples contained only **dapoxyl-F** (0.5 μM). All samples were incubated at room (22.5ºC) temperature. Fluorescent spectra were recorded after indicated incubation times. Data of three independent experiments were processed using Microsoft Excel.

NASBA. Viral RNA, 50 fg/μL (final concentration) was added to 1×NASBA reaction buffer NASBA buffer (TrisHCl, pH 8.5 at 25 ºC, $MgCl_2$, KCl, DTT, and DMSO; Life Sciences Advanced Technologies Inc.), 1× nucleotide mix (NECN-1-24) 250 nM NASBA revers and forward primers (Table SI 1). The total volume of each reaction mixture was adjusted to 12 μL with water. Samples were incubated at 65 ºC for 2 min followed by cooling to 41 ºC for 10 min. Three microliters of NASBA enzyme cocktail (AMV RT, RNase H, T7 RNAP, BSA, high MW sugar matrix; Life Sciences Advanced Technologies Inc.) was added and samples were incubated at 41 ºC for 90 min. Samples were analyzed in 2% Agarose gel (Figure 2B). The concentration of Z-147 amplicon was estimated based on the intensity of the correspondent band in gel and comparison with the intensity of the 200 nt band of RiboRuler Low Range RNA Ladder (ThermoFisher Scientific)

Analysis of NASBA amplicon by SDA probe. Time drive experiments were performed in NASBA buffer at room temperature (~22 ºC) using a Cary Eclipse Fluorescence Spectrophotometer in a kinetic mode. The samples contained 0.5 μM. **Dapoxyl-F** (0.5 μM), **SDA** (0.5 μM) **SDA_f2** (4 μM) and either 10% NASBA amplicon Z-147 or 10% NASBA no-target control. Blank contained **dapoxyl-F** (0.5 μM), **SDA_m2** (0.5 μM) **SDA_f2** (4 μM) in the absence of an analyte. As a positive control, a sample containing 100 nM **Z-64** synthetic DNA analyte instead of NASBA amplicon was used.

4. *Figure S1. Detailed design of SDA and comparison with full DAP-10 aptamer*

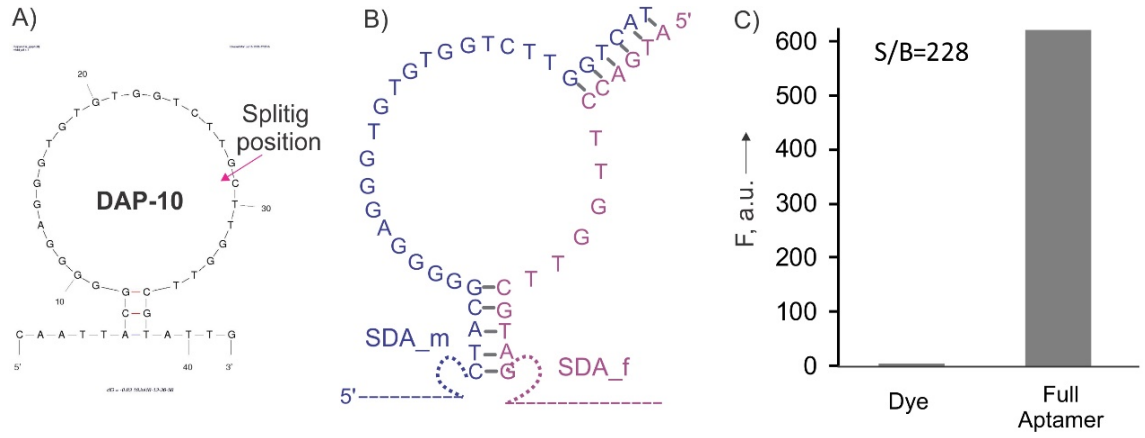

Figure S1. A) The structure of **DAP-10**[1] predicted by mfold[2] under the assay conditions (22.5°C, 10 mM $MgCl_2$, 200 mM K^+). The split position is indicated by red arrow. B) Design of SDA. Dashed lines indicate binding arms; Dotted lines indicate linkers/ Constructs used in this study differed by the analyte binding arms and the linkers. C) Fluorescence Turn-on properties f full **Dap-10**. The samples contained 0.5 μM of **dapoxyl-F** (Dye), or 0.5 μM of **dapoxyl-F** and 1 μM DAP-10 in Buffer 1. Fluorescent measurements were taken after 30 min of incubation (λ_{ex} = 385 nm, λ_{em} = 505 nm). The data are average values of 3 independent measurements with a single standard deviation.

5. *Figure S1. Dapoxyl-F H-NMR Data*

Dapoxyl-F dye was synthesized according to the publish procedure.[3] It was characterized by H^1 NMR, which confirmed the structure of **Dapoxyl-F** shown in Figure S 2.

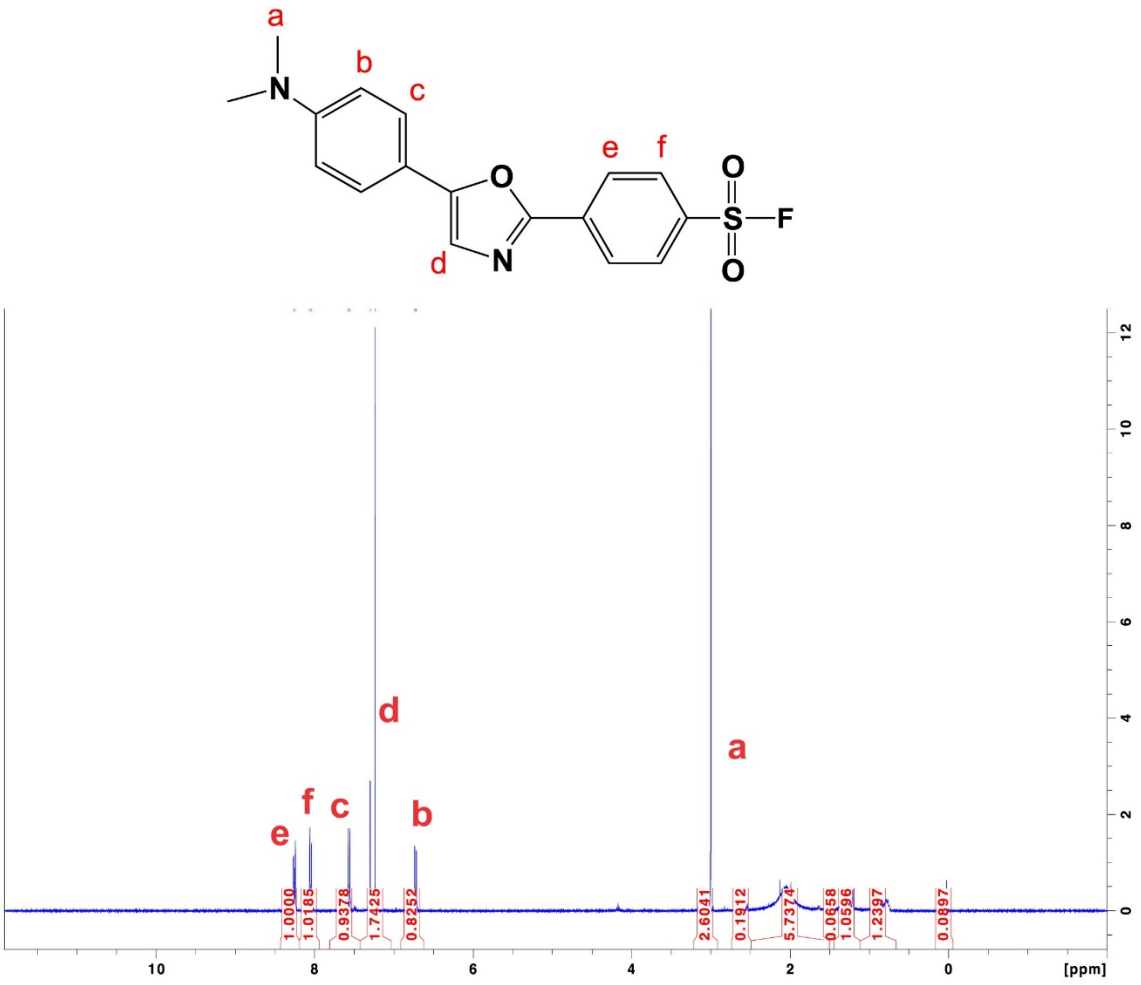

Figure S2. ^{1}H NMR spectrum and signal assignment for **dapoxyl-F** dye. The letters represent peak assignment for each group of hydrogen atoms.

6. *Figure S3. Suboptimal designs of SDA*

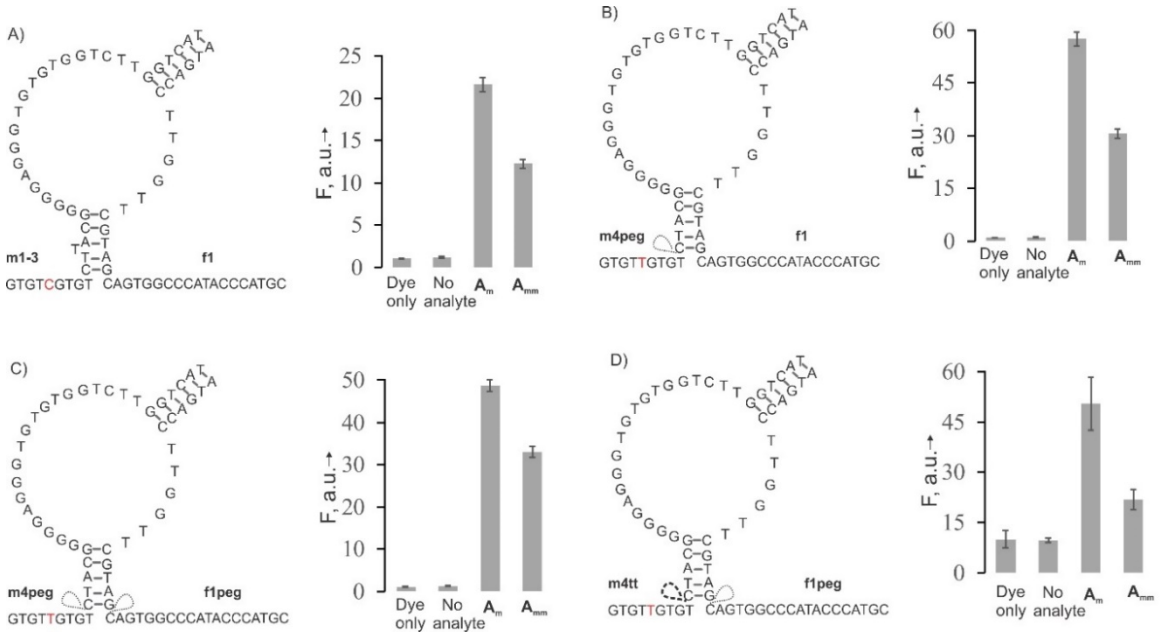

Figure S3. Suboptimal designs of SDA. A-D) Four different combinations of linkers for each SDA probe targeting A_m analyte were tested to select designs with the greatest turn-on ratio and ability to differentiate $\mathbf{A_m}$ from $\mathbf{A_{mm}}$. The fluorescence data for each combination is shown on the right next to each design. Linker is indicated in gray dot, triethylene glycol; bold dot, dithymidine. The data are average values of 3 independent measurements with a single standard deviation.

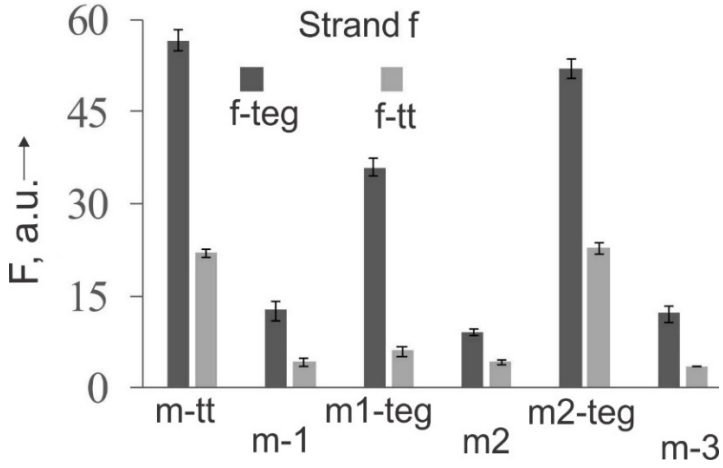

Figure S4. Optimization of the linker between the aptameric sequence and analyte-binding arm in SDA_f and SDA_m: teg, triethylene glycol linkers; tt, dithymidine linker. Samples contained 1 μM SDA_f, 1 μM SDA_m, and 0.5 μM **dapoxyl-F** in Buffer 1 (20 mM Tris-Cl, pH 7.4, 200 mM KCl, 10 mM $MgCl_2$) in the presence of 1 μM $\mathbf{A_m}$ analyte. Light grey and dark grey bars indicate triethylene glycol (teg) and dithymine (tt) linker-containing SDA_f, respectively. The data of 3 independent experiments was averaged, with standard deviations as error bars. Fluorescence was taken after 40 min of incubation. Refer to Supplemental Information (Table SI 1) for the strand sequences.

7. *Figure S5. Limit of detection for SDA with dapoxyl-F*

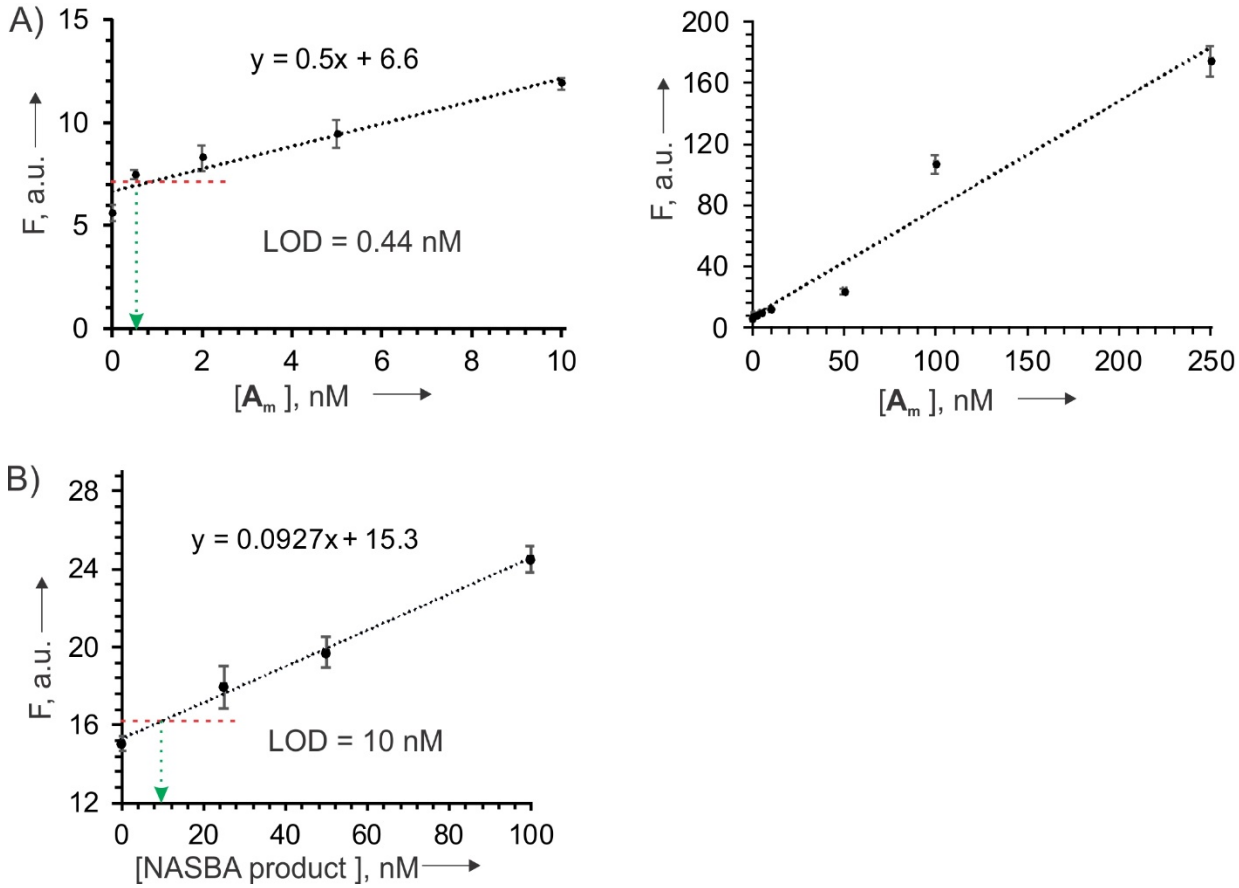

Figure S5. Limit of detection (LOD) for SDA. A) LOD for detection of $\mathbf{A_m}$ using SDA1 probe. The samples contained **Dapoxyl-F** (0.5 μM) **SDA_m1** (1 μM) and **SDA_f1** (0.2 μM) strands in Buffer 1 in the absence or presence of different concentrations (0, 0.5, 2, 5 and 10 nM) of $\mathbf{A_m}$ analyte. The LOD was calculated from the left panel, the right panel represents broader concentration range. B) LOD for NASBA product was measured using SDA-2 sensor as described above for SDA-1 sensor. NASBA product was quantified on 2% agarose gel stained with GelRed by comparison of relative intensities to 100 nt. band in RiboRuler low range RNA ladder (Thermo Fisher Catalog number SM1831). The samples contained 0.5 μM **dapoxyl-F,** 4 μM **SDA_f**, 1.0 μM **SDA_m**, and analyte **(NASBA amplicon**) in Buffer 1 (20 mM Tris-HCl, pH 7.4, 200 mM KCl, 10 mM $MgCl_2$). The LOD was found to be 10 nM. All readings were taken after 60 min of incubation. The data are average values of 3 independent measurements with a single standard deviation. LOD was calculated as a concentration that produce a signal above the background plus three standard deviations according to the ACS recommended procedure.[4]

8. *Figure S6. SDA fluorescence data for Z-64*

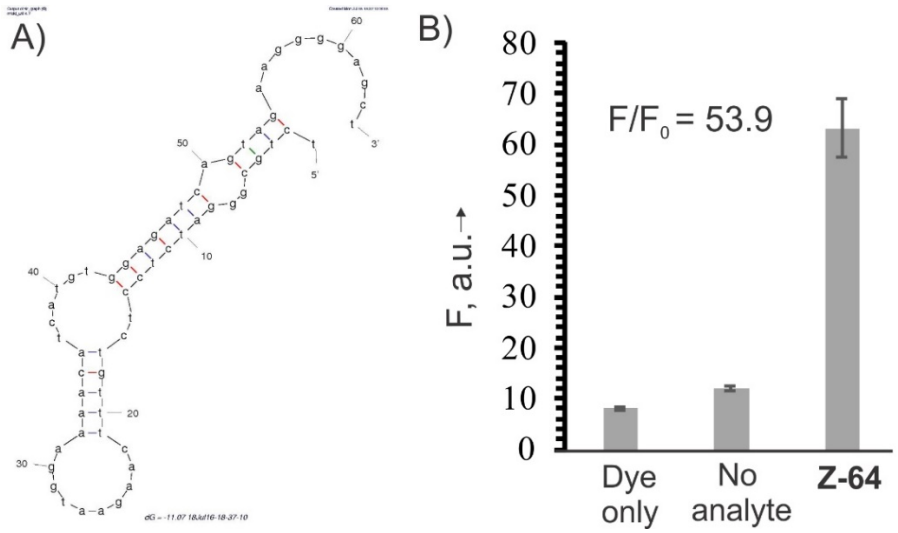

Figure S6. SDA Fluorescence data for SDA-3 targeting **Z-64**. A) Secondary structure of Z-64 predicted by mfold. B) Performance of SDA2 towards Z-64. The samples contained 0.5 µM **dapoxyl-F** (dye only), 0.5 µM dapoxyl-F, 0.5 µM **SDA_f2**, 4 µM **SDA_m2** (No analyte) and 0.5 µM **dapoxyl-F**, 0.5 µM SDA_m4, 4 µM SDA_f4, and 0.5 µM of matched analyte (**Z-64**) in NASBA buffer.

9. *Figure S7. Comparison of SDA responses at 37 and 22.5°C.*

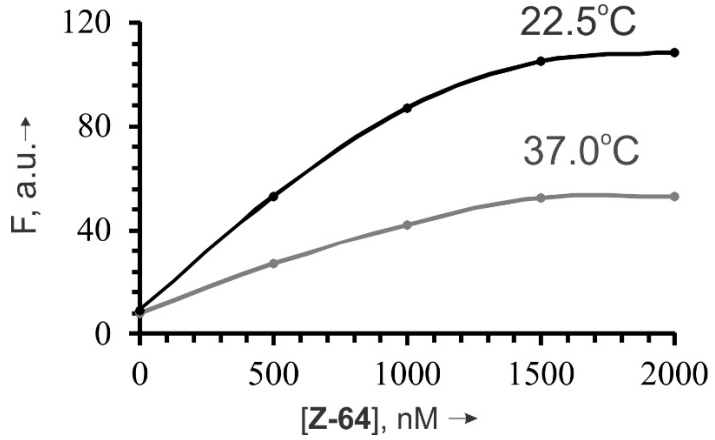

Figure S7. Dapoxyl fluorescence performance at elevated temperature. The samples contained 0.5 µM **dapoxyl-F,** 3 µM **SDA_f**, 1.25 µM **SDA_m**, and analyte **(Z-64**) in **NASBA buffer** at various temperature indicated above the line plot. All measurements were taken after 60 min of incubation. The data are average values of 3 independent measurements with a single standard deviation.

10. References

1. Kato, T.; Shimada, I.; Kimura, R.; Hyuga, M., Light-up fluorophore-DNA aptamer pair for label-free turn-on aptamer sensors. *Chem. Com*. 2016, 52, 4041-4044.

2. Zuker, M. Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res. 2003, 31, 3406-3415.

3. Diwu, Z.; Lu, Y.; Zhang, C.; Klaubert, D. H.; Haugland, R. P., Fluorescent Molecular Probes II. The Synthesis, Spectral Properties and Use of Fluorescent Solvatochromic Dapoxyl Dyes. *Photochem. Photobiol.* **1997,** *66*, 424-431.

4. MacDougall, W. B. Crummett et al. Guidelines for data acquisition and data quality evaluation in environmental chemistry *Anal. Chem.* **1980**, *52*, 2242– 2249.