Supporting Information

Promiscuous dye binding by a light-up aptamer: Application for label-free multi-wavelength biosensing

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Experimental details

Materials. Oligonucleotides were custom-synthesized by IDT, Inc. (Coralville, IA, USA). Auramine O and brilliant green were purchased from MP Biomedicals (Solon, OH, USA). Basic fuchsin and Michler's ketone were purchased from Alfa Aesar (Ward Hill, MA, USA). Crystal violet and malachite green were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fluorescein, methyl violet 2B, pararosaniline, thioflavin T, and victoria blue B were purchased from ACROS Organics (Morris, NJ, USA). *N*-methyl mesoporphyrin IX was purchased from Frontier Scientific (Logan, UT, USA). Rhodamine 6G was from Armor Forensics (Jacksonville, FL, USA). Quartz cuvettes for measuring absorbance, fluorescence, or circular dichroism were purchased from Starna Cells (Atascadero, CA, USA). NASBA liquid kit was purchased from Life Sciences Advanced Technologies Inc. (Saint-Petersburg, FL). RiboRuler Low Range RNA Ladder was from ThermoFisher Scientific (Waltham, MA). Agarose was from Lonza (Basel, Switzerland).

Characterization of dapoxyl sulfonyl fluoride (DSF). Dapoxyl sulfonyl fluoride (DSF) was synthesized as previously described[1] and kindly provided by Dr. Kikuchi. The dye sample was characterized prior to use in this work. ESI-MS: $[M+H]^+_{calculated}$=347.09, $[M+H]^+_{observed}$=347.08. ^{1}H-NMR ($CDCl_3$): 8.30 (2H, d); 8.09 (2H, d); 7.61 (2H, d); 7.34 (1H, s); 6.77 (2H, d); 3.05 (6H, s). ^{19}F-NMR ($CDCl_3$): 66.26 (s).

Affinity constant determination. Samples contained 0-50 µM DAP-10-42 and 2 µM of the respective dye. DSF, AO, and MK were suspended in buffer A (20 mM Tris-HCl, pH 7.4, 20 mM KCl, and 25 mM $MgCl_2$); all other dyes were measured in buffer B (50 mM HEPES, pH 7.4, 20 mM KCl, 50 mM $MgCl_2$, 120 mM NaCl, 1% DMSO, and 0.03% Triton X-100). Fluorescence data was blank subtracted then normalized to the largest value in range as 100% Turn-On (TO%). Excitation/emission wavelengths were as follows: DSF, 390/507 nm; TFT, 440/500 nm; CV and MV, 590/630 nm; MG and BG, 617/660 nm; PR, 557/600 nm; BF, 560/600 nm; VB, 635/690 nm; AO, 475/540 nm; MK, 390/450 nm. Fluorescence intensity was plotted as a function of the aptamer concentration ([DAP]) and analyzed using Origin software. The K_d values were calculated using an equation below:

$$S/B = \frac{(A_T + D_T + K_d) - \sqrt{(A_T + D_T + K_d)^2 - 4A_T D_T}}{2A_T}$$

where S/B is the signal-to-background ratio calculated by dividing the fluorescence intensity of the aptamer-containing sample by the average intrinsic dye's fluorescence, A_T is the total concentration of DAP-10-42 in a given sample and D_T=2 μM is the total dye concentration.

Quantum yield analysis. Samples containing 0-7.5 μM DAP-10-58 and 7.5 μM AO, or 7.5 μM DAP-10-58 and 0-7.5 μM AO, were prepared in the buffer A. Standards contained fluorescein in 0.1 M sodium hydroxide or rhodamine 6G in ethanol. Absorbance spectra were collected with a NanoDrop One^C (ThermoScientific, Waltham, MA, USA) at ambient temperature using a 1 cm quartz cuvette. Fluorescent spectra were recorded upon excitation at 475 nm using a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at ambient temperature using a 3 mm quartz cuvette.

Job plot analysis. Samples were prepared in the assay buffer (20 mM Tris-HCl, pH 7.4, 20 mM KCl, 25 mM $MgCl_2$) at 4 μM total molar concentration of both DAP-10-42 and either DSF or auramine O (AO). Fluorescence emission was measured using a Cary Eclipse Spectrofluorimeter at 507 nm upon excitation at 399 nm (DSF), or at 540 nm upon excitation at 475 nm (AO) in a 1 cm quartz cuvette.

SDA probe assay. Samples (50 μl) containing SDA-S and SDA-U (each at 0.5 μM) in buffer A supplemented with 1% DMSO and 2 μM dye (DSF, AO, CV, or TFT) in the presence of a synthetic target (katG or katG-G>C) at the indicated concentration or 2% (v/v) NASBA sample were incubated at 22°C for 15 min prior to measuring their fluorescence using a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at ambient temperature using a 1 cm quartz cuvette. Alternatively, fluorescence measurements were performed in a time-drive mode using an Infinite® 200 PRO Plate Reader (Tecan, Switzerland). The measurements in this case began within 5-10 min of the target addition. As a negative control, a sample in the absence of the target was used.

Calculation of LOD and LOQ was performed using a 3σ/S and 10σ/S rule,[2] respectively, where σ is the standard deviation of the *y*-axis intercept, and S is the slope of the linear trendline for the signal concentration dependence in the linear dynamic range. The data processing was done using OriginLab© 2018b graphing software.

NASBA reaction was performed according to the manufacturer's instruction, with the sample volume decreased to 12 μl. Specifically, samples were prepared by mixing 4 μl of 3×NASBA Reaction Buffer, 2 μl of 6×Nucleotide Mix, 2 μl of 1.5 μM primer mix, and 1 μl of either RNase-free water (for no-target control, NTC) or total bacterial RNA (10 pg/μl). The samples were incubated at 65 °C for 2 min followed by cooling to 41 °C for 10 min. Then, 3 μl of 4×NASBA enzyme cocktail was added, and the samples were incubated at 41 °C for 90 min. The obtained samples were analyzed by 2% agarose gel electrophoresis (1 μl per lane) with RNA in the gel stained by GelRed. Amplicon concentration in the NASBA samples was determined by comparing the intensity of the corresponding band with the intensity of the band containing a 300-nt marker of the RiboRuler Low Range RNA ladder using a BioRad GelDoc XR+ Molecular Imager coupled with ImageLab software.

Table S1. ***Sequences of oligonucleotides used in this study.***

Name	**Sequence, 5'→3'**[a]
ssDNA	GGACATTTTAGAACATTACATACCCCG
slDNA	GGACCAGAACAACCCGCTGTCGGGGTTGACCGACAAGCGCCG
TBA	GGTTGGTGTGGTTGG
PW17	GGGTAGGGCGGGTTGGG
CV30S	AACGACCACCGGTGCGCCGTACAGGTAACTAGCGTCGTCGTT
Tel23a	AGGGTTAGGGTTAGGGTTAGGGT
MG1-3	CTCAGATCTAACCTTGTTAAATTGAG
DIR2-1	GACGACGACGCTAGGAAGGCGTTGGTGGGCACGCCGGTCGTC
AT11	TGGTGGTGGTTGTTGTGGTGGTGGTGGT
EAD2	CTGGGAGGGAGGGAGGGA
Agro100	GGTGGTGGTGGTTGTGGTGGTGGTGG
HT	AGGGTTAGGGTTAGGGTTAGGG
RHT	GGGTTAGGGTTAGGGTTAGGGA
DAP-10-42	CAATTACG*GGGGAGGGTGTGTGGTCTTGCTTGGTT*CGTATTG
SDA-U	TTCGTCCATACGACCTCGATGTTCTACG*GGGGAGGGTGTGTGGTTTT*GGTCAT
SDA-S	ATGACC*TTGGTT*CGTAGTTCCG**C**TGGTG
katG	AACCGGTAAGGACGCGATCACCA**G**CGGCATCGAGGTCGTATGGACGAACACCCCGACGAAATGGGACAACAGTTTCCTCG
katG-G>C	AACCGGTAAGGACGCGATCACCA**C**CGGCATCGAGGTCGTATGGACGAACACCCCGACGAAATGGGACAACAGTTTCCTCG
NASBA-katG	gggagaaggcuugggcuggaagagcucguauggcaccggaaccgguaaggacgcgaucaccag**g**cggcaucgaggucguauggacgaacaccccgacgaaaugggacaacaguuuccucgagauccuguacggcuacgagugg

[a]Stem-forming nucleotides are underlined; nucleotides of the dye-binding aptamer core are in italics "linker" nucleotides in the sensor strands are in grey; single-nucleotide substitution positions in katG and katG-Mut are in bold; complementary nucleotides of the sensor strands and the targets are color-coded; ribonucleotides in the NASBA amplicon of the *katG* gene are in lowercase, with nucleotides absent in the mRNA indicated in grey lowercase.

Table S2. Characteristics of DAP-10-42 interaction with different fluorogenic dyes.

Dye	K_d (µM)	S/B_{MAX}[a]	LOD[b] (nM)	Dye	K_d (µM)	S/B_{MAX}[a]	LOD[b] (nM)
DSF	0.011 ± 0.022	132	5.4	**PR**	24 ± 1.6	18	N/D
CV	0.21 ±0.07	131	4.7	**BF**	5.9 ± 1.2	20	N/D
MV	0.42 ± 0.05	104	N/D	**VB**	3.4 ± 2.2	2	N/D
MG	6.9 ± 0.82	258	N/D	**AO**	0.74 ± 0.12	2070	2.6
BG	4.8 ± 0.87	59	N/D	**MK**	6.6 ± 0.89	6	N/D
TFT	10 ± 1.1	1870	2.8	**NMM**	130 ± 79	121	N/D

[a]The signal-to-background ratio (S/B_{MAX}) was determined from the fitting of the data in Figure 1B using the Origin software. It serves as a measure of the dyes' fluorescence turn-on.

[b]The LOD values are determined for the SDA probe in the presence of the selected dyes. N/D – not determined.

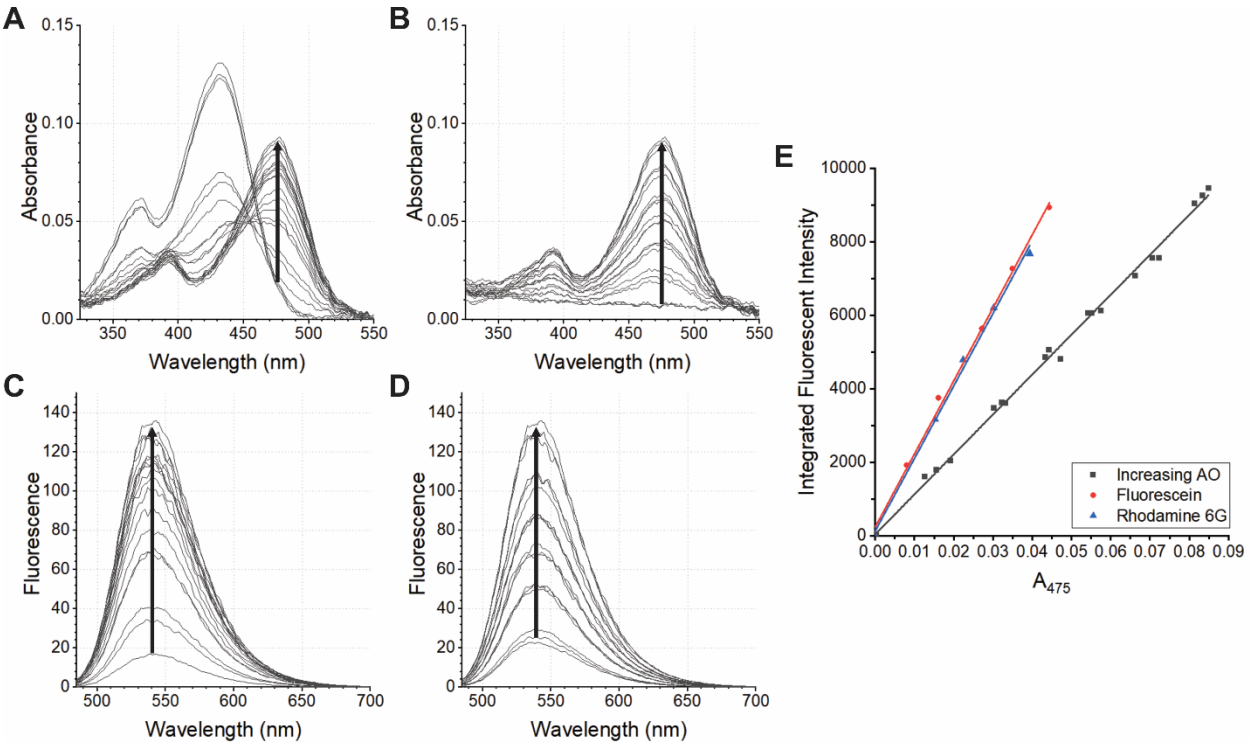

Figure S1. ***Quantum yield*** *(*ϕ_f) determination for the DAP-10-42:AO complex. Absorbance spectra for increasing concentrations (0-7.5 µM) of DAP-10-42 at 7.5 µM AO (A) and increasing concentrations (0-7.5 µM) of AO at 7.5 µM DAP-10-58 (B), as well as their respective fluorescence emission spectra upon excitation at 475 nm (C and D, respectively) were recorded in the assay buffer. Fluorescent spectra are shown after 7-point Savitzky-Golay smoothing. The slopes of integrated fluorescent intensity versus A_{475} for each set (E) were compared to two standards (fluorescein, rhodamine 6G) with literature ϕ_f values of 0.95 and experimental ϕ_f values of 0.934 and 0.966, respectively. The average ϕ_f compared to the two standards is 0.511.

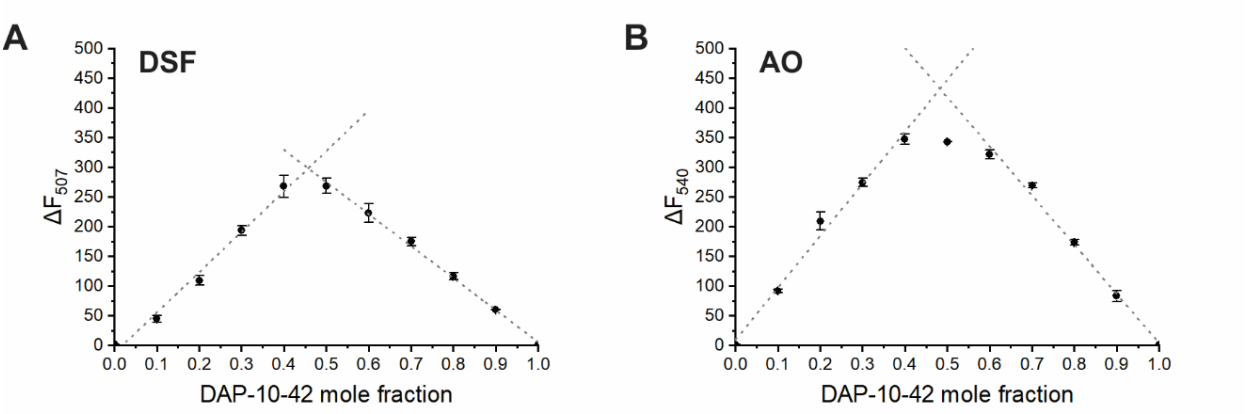

Figure S2. ***Continuous variations analysis*** (Job plot) for the complex of DAP-10-42 with DSA (A) or AO (B). The total concentration of the dye and aptamer was 4 µM. The DAP-10-42 molar fraction was calculated as the ratio between the aptamer concentration and the total (aptamer and dye) concentration. Average values from three independent experiments are shown with the error bars as standard deviations.

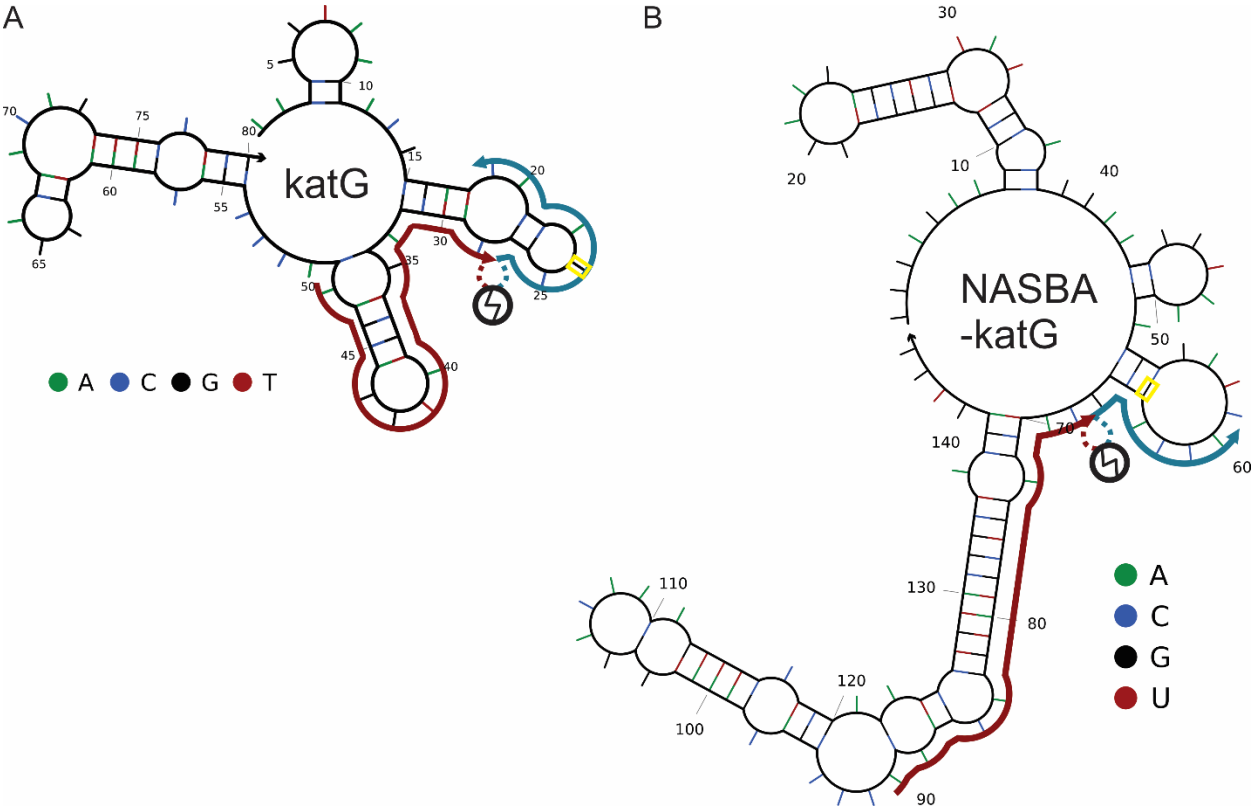

Figure S3. ***Minimum energy secondary structures*** of the synthetic target katG (A) and NASBA amplicon NASBA-katG (B) as predicted by NUPACK software (http://www.nupack.org/). The G315C mutation site is outlined with a yellow box. The nucleotide identity is color-coded. The target fragments interacting with SDA-S and SDA-U are indicated with cyan and red lines, respectively.

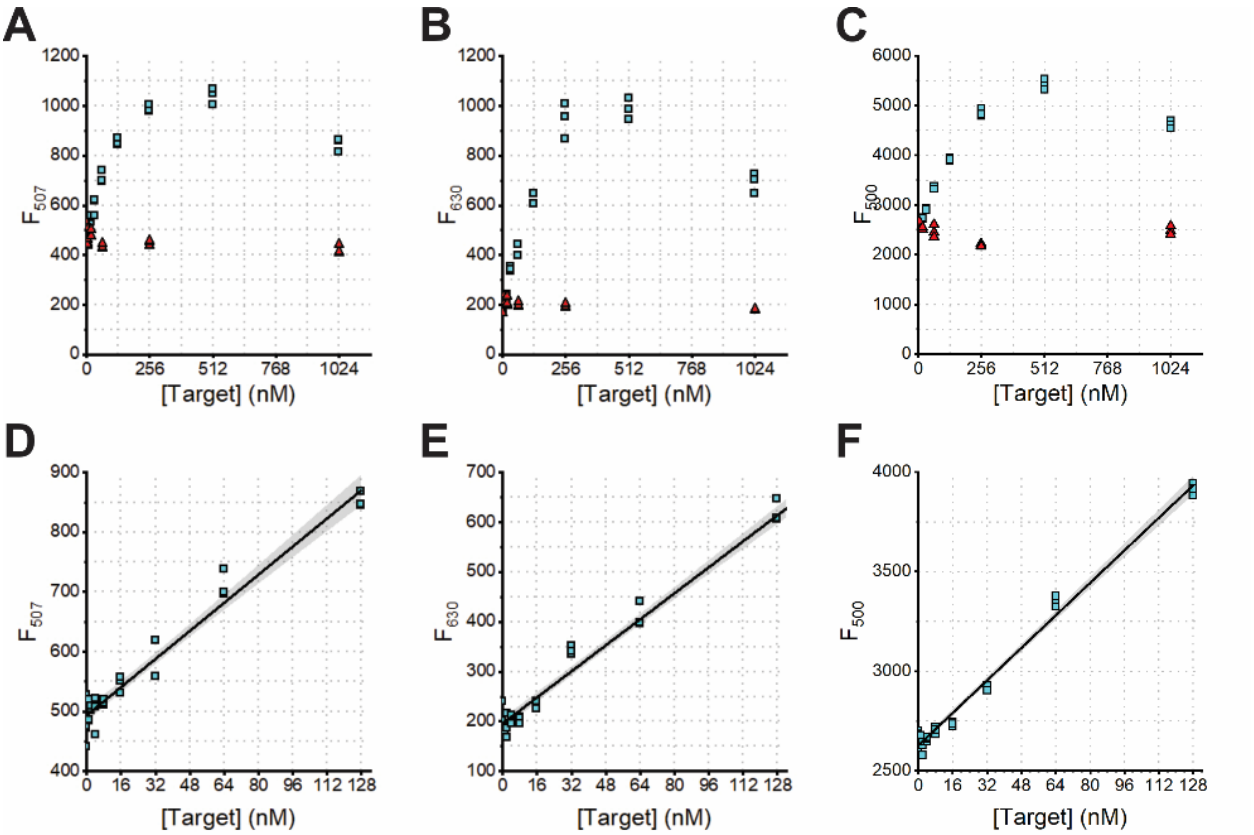

Figure S4. ***Response of the SDA probe*** utilizing DSF (A and D), CV (B and E), or TFT (C and F) to increasing concentrations (0-1024 nM) of katG (cyan squares) or katG-G>C (red triangles). Response to katG within the linear dynamic range (D-F) was used to calculate the limits of detection and quantification using the 3σ and 10σ rules, respectively. The calculated values are listed in Table 1. The line of best fit and 95% confidence interval are shown (black line, gray shading). For each graph shown, the fluorescence intensity values from three independent experiments were combined in one plot.

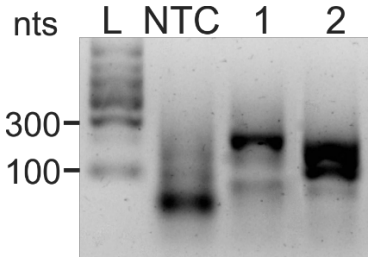

Figure S5. ***Analysis of NASBA samples in 2% agarose gel electrophoresis.*** NASBA no-target control (NTC) and two amplicon-containing samples (1 and 2) obtained in parallel by two different experimentalists using total RNA from *M. tuberculosis* (10 pg per reaction) were loaded on the gel in aliquots of 1 μl. L – RiboRuler Low Range RNA ladder (70 ng/μl per band, 0.5 μl per lane).

References

1. Diwu, Z., Lu, Y., Zhang, C., Klaubert, D. H., Haugland, R. P. (1997) *Photochem. Photobiol.* 66, 424-431.
2. MacDougall, D., Crummett, W. B. (1980) *Anal. Chem.* 52, 2242–2249.