Supporting Information

Cascade of Deoxyribozymes for the Colorimetric Analysis of Drug Resistance in *Mycobacterium tuberculosis*

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Content

Table S1. Sequences of the probes and targets used in the study.[a]

Gene	Name	Sequence (5'-3')
N/A	**IPDz**	GGGTAGGGCGGGTTGGGTTC**gu**CCATGAGCAACTCGCCC
rrs	**16S_MTC**	ACTGGGTCTAATACCGGATAGGACCACGGGATGCATGTCTTGTGGTGGAA
	16S_Mab	ACTGGGTCTAATACCGGATAGGACCACACACTTTCATGGTGACTGGTGCAA
	16S_NASBA_MTC	GGGAGAAGGAGCCUGGGAAACTGGGUCUAAUACCGGAUAGGACCACGGGAUGCAUGUC UUGUGGUGGAAAGCGCUUUAGCGGUGTGGGAUGAGCC
	S1_16S_MTC	GTTGCTCATGGA*GGCTAGCT*GGTCCTATCCGGTATTAGACCC
	S2_16S_MTC	CACAAGACATGCATCCCGT*ACAACGA*GAACCCAACC
katG	**kG_INH^S**	GCTGGAAGAGCTCGTATGGCACCGGAACCGGTAAGGACGCGATCACCAGCGGC
	kG_INH^R	GCTGGAAGAGCTCGTATGGCACCGGAACCGGTAAGGACGCGATCACCACCGGC
	kG_NASBA_INH^S	GGGAGAAGGGCUUGGGCUGGAAGAGCUCGUAUGGCACCGGAACCGGUAAGGACGCGAU CACCAGCGGCAUCGAGGUCGUAUGGACGAACACCCCGACGAAAUGGGACAACAGUUUCC UCGAGAUCCUGUACGGCUACGAGUGG
	U_kG	GTTGCTCATGGA*GGCTAGCT*GCGTCCTTACCGGTTCCGGTGCCAT
	S_kG_INH^S	GCCGCTGGTGATC*ACAACGA*GAACCCAACC
	S_kG_INH^R	GCCGGTGGTGATCC*ACAACGA*GAACCCAACC
rpoB	**rB_RIF^S**	GGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCG
	rB_T_RIF^R	GGACCAGAACAACCCGCTGTCGGGGTTGACCTACAAGCGCCG
	rB_G_RIF^R	GGACCAGAACAACCCGCTGTCGGGGTTGACCGACAAGCGCCG
	U_rB	GTTGCTCATGGA*GGCTAGCT*CCCGACAGCGGGTTGTTCTGGTCC
	S_rB_RIF^S	GCTTGTGGGTCAAC*ACAACGA*GAACCCAACC
	S_rB_T_RIF^R	GCTTGTAGGTCAAC*ACAACGA*GAACCCAACC
	S_rB_G_RIF^R	GCTTGTCGGTCAAC*ACAACGA*GAACCCAACC
gyrA	**gA_FQ^S**	GGCGACGCGTCGATCTACGACACCCTGGTGCGCATGGCCCAGCCCTGGTCGC
	gA_FQ^R	GGCGACGCGTCGATCTACGGCACCCTGGTGCGCATGGCCCAGCCCTGGTCGC
	U_gA	GCGACCAGGGCTGGGCCATGCGCACC*ACAACGA*GAACCCAACC
	U2_gA	GCGACCAGGGCTGGGCCATGCGCACC**AG***ACAACGA*GAACCCAACC
	S_gA_FQ^S	GTTGCTCATGGA*GGCTAGCT***AG**GGTGTCGTAGA
	S2_gA_FQ^S	GTTGCTCATGGA*GGCTAGCT*GGTGTCGTAGA
	***s*/S_gA_FQ^S**	GTTGCTCATGGA*GGCTAGCT***AG**GGTGTCGTAGAGACAC
	S_gA_FQ^R	GTTGCTCATGGA*GGCTAGCT***AG**GGTGCCGTAGATC
	S2_gA_FQ^R	GTTGCTCATGGA*GGCTAGCT*GGTGCCGTAGA
	***s*/S_gA_FQ^R**	GTTGCTCATGGA*GGCTAGCT***AG**GGTGCCGTAGACACC

[a]Nucleotides at the probed SNS positions, as well as of the target-recognizing elements complementary to the target SNS sites, are in magenta. Target fragments interrogated by strand U of the correspondent probes, as well as the target-binding fragments of strand U, are underlined. Nucleotides in the targets not interacting with the probes, as well as nucleotides adjacent to the target-binding fragments of the sIS strands that not complementary to the targets, are in grey. Complementary nucleotides in *s*/S strands are underlined. Nucleotides of the Dz catalytic core are in italics Nucleotides of the IPDz-binding fragments of the probe strands, as well as the complementary IPDz fragments, are in green. Ribonucleotides in the IPDz reporter are in lowercase.

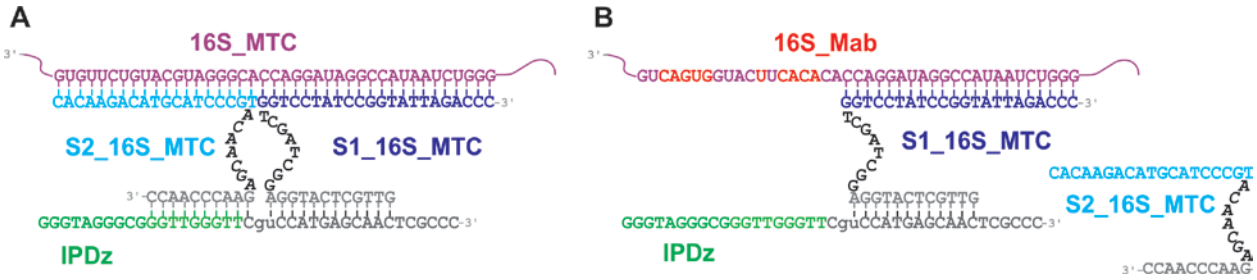

Figure S1. Complexes of the sDz/PDz probe targeting a fragment of MTC 16S rRNA with the IPDz reporter and either specific (A) or non-specific (B) target. As the non-specific target, a fragment of 16S rRNA from *M. avium* (*Mab*) is shown. The two targets differ by several nucleotides in the fragment interacting with one of the probe's strands (S2_16S). The sequences are listed in Table S1. The IPDz fragment released upon IPDz cleavage in the presence of the specific target is in green. The nucleotides of the Dz catalytic core are in black.

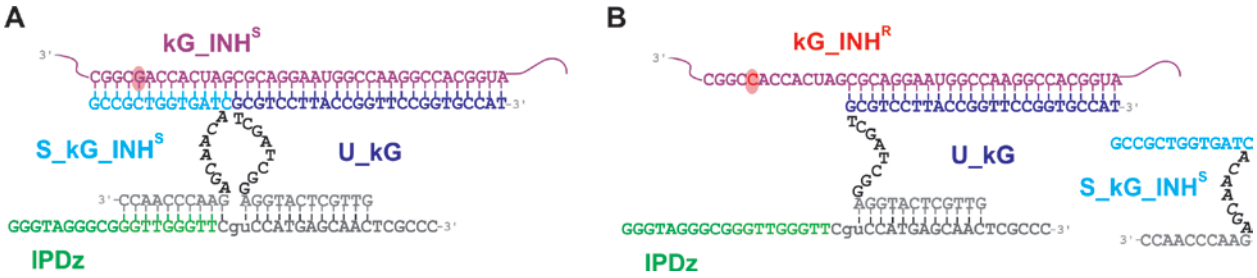

Figure S2. Complexes of the sDz/PDz probe targeting a fragment of the *katG* gene sensitive to isoniazid (INH^S) with the IPDz reporter and either specific (A) or non-specific (B) target. The two targets differ by a single nucleotide in codon 315 of the gene. The position of the single-nucleotide substitution (SNS) is highlighted with a red oval. The SNS-bearing fragment of the targets is interrogated by strand S of the probe ($S\_kG\_INH^S$), which binds only to the fully complementary target. The sequences are listed in Table S1. The IPDz fragment released upon IPDz cleavage in the presence of the specific target is in green. The nucleotides of the Dz catalytic core are in black.

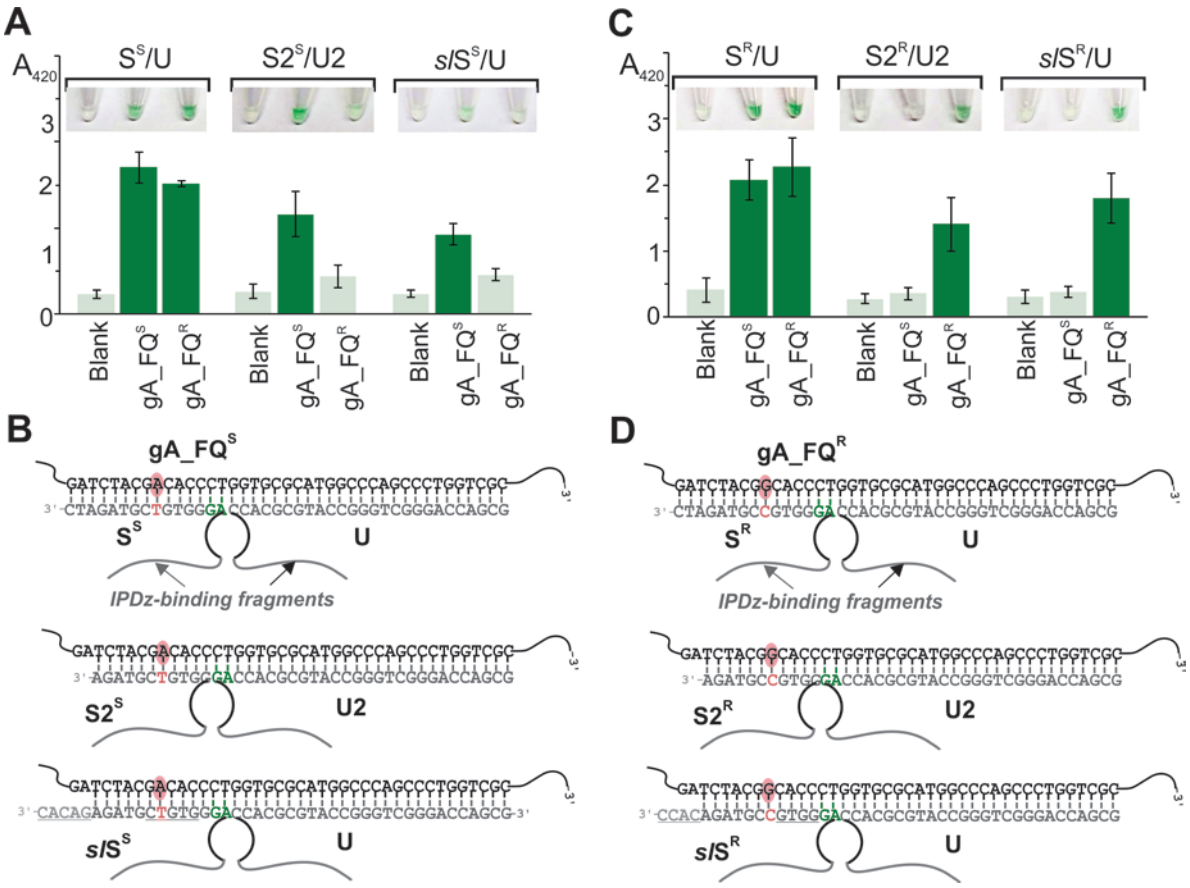

Figure S3. Selectivity of the sDz/PDz probes targeting a fragment of the *gyrA* gene. A and B. The probes specific for gA_FQ^S target contained the indicated "unwinding" strands U_gA or U2_gA, and target-differentiating strands S_gA_FQ^S (S^S), S2_gA_FQ^S (S2^S), or *sl*S_gA_FQ^S (*sl*S^S). **C and D.** The probes specific for gA_FQ^R target contained the indicated "unwinding" strands U_gA or U2_gA, and target-differentiating strands S_gA_FQ^S (S^R), S2_gA_FQ^R (S2^R), or *sl*S_gA_FQ^R (*sl*S^R). **A and C.** Response of the indicated probes in the absence (NTC) or presence of either specific or non-specific target. **B and D.** Complexes of the targets with the probe strands.

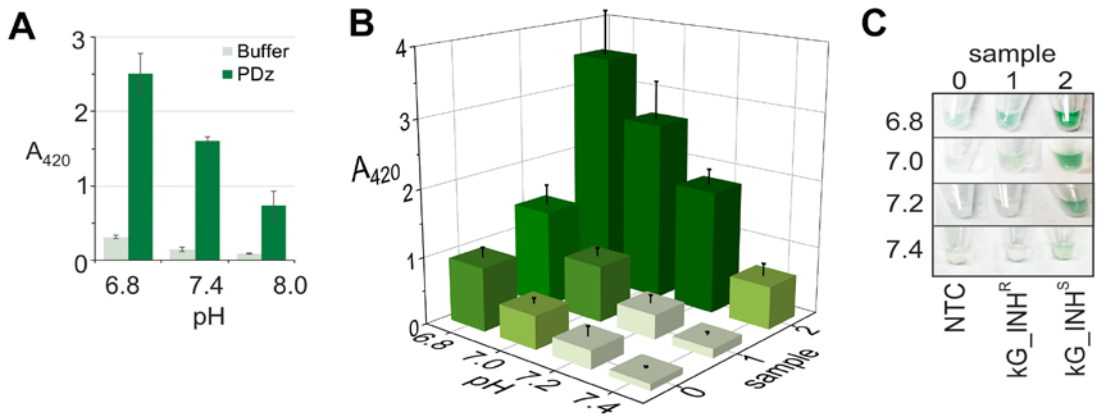

Figure S4. Performance of the sDz/PDz system as a function of pH. A. Absorbance at 420 nm of the samples containing the assay buffer supplemented with hemin (375 nM), ABTS (1 mM) and H_2O_2 (1 mM) in the absence and presence of PDz (500 nM) at various pH of the buffer. **B.** Absorbance at 420 nm of the samples containing all the components of the sDz/PDs system targeting kG_INH^S in the assay buffer at various pH. The samples contained no target (sample 0), or 100 nM kG_INH^S (sample 1) or kG_INH^R (sample 2). **C.** Tube images of the samples used for panel **B**.

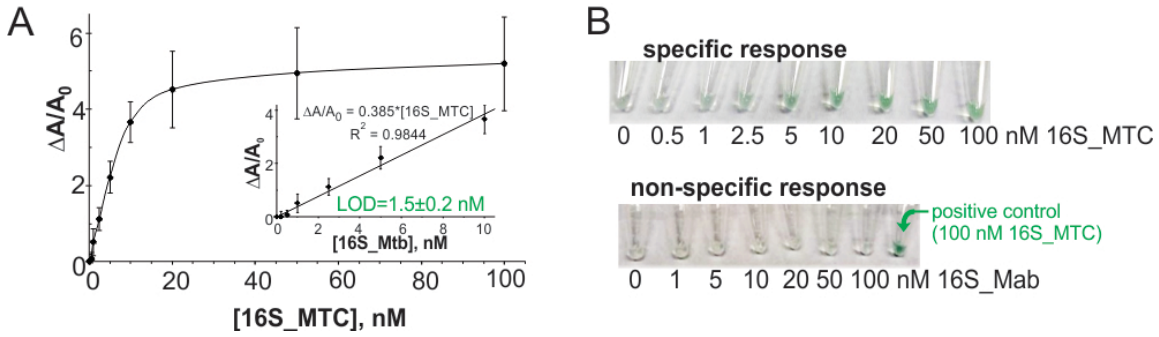

Figure S5. Performance of the sDz/PDz probe interrogating a fragment of MTC 16S rRNA. A. Signal for the samples containing IPDz (1 μM), and S1_16S_MTC and S1_16S_MTC (100 nM each) as a function of 16S_MTC concentration. as $\Delta A/A_0$, where $\Delta A=A-A_0$, and A_0 and A are absorbance values for the samples in the absence of presence of the target, respectively. *Inset:* linear dependence of the signal on the target concentration (0-10 nM) and the limit of detection. **B.** Visually observed signal triggered by either specific (16S_MTC, top) or non-specific (16S_Mab, bottom) target at the indicated concentrations. In the bottom panel, the last tube contains the MTC-specific sDz/PDz probe in the presence of 16S_MTC target (100 nM) to serve as a positive control for the colorimetric assay.

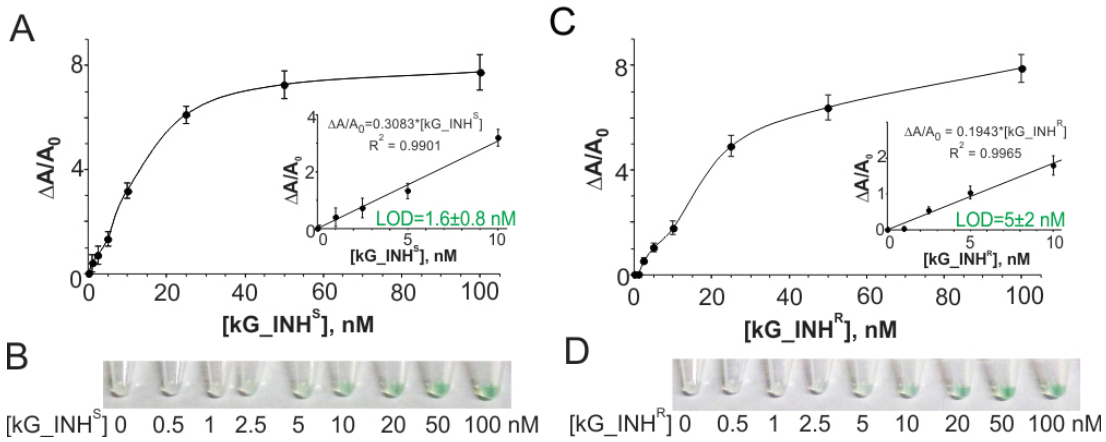

Figure S6. Performance of the sDz/PDz probes interrogating a fragment of the *katG* gene. Response of the kG_INH^S-specific sPDz probe as a function of kG_INH^S concentration **(Panels A and B)**. Response of the kG_INH^R-specific sPDz probe as a function of kG_INH^R concentration **(Panels C and D)**. **Panels A and C:** The signal is plotted as $\Delta A/A_0$, where $\Delta A=A-A_0$, and A_0 and A are absorbance values for the samples in the absence of presence of the target, respectively. *Inset:* linear dependence of the signal on the target concentration in the range of 0-10 nM and the limit of detection (LOD). **Panels B and D:** Visually observed signal triggered by the correspondent specific target at the indicated concentrations.

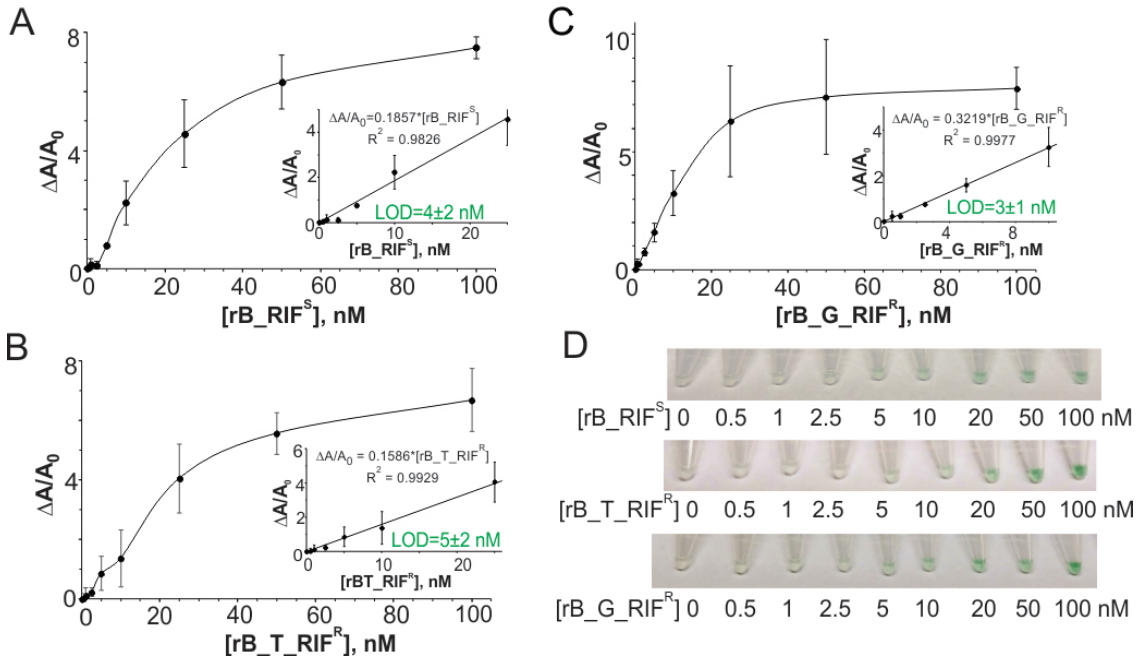

Figure S7. Performance of the sDz/PDz probes interrogating a fragment of the *rpoB* gene. A: Response of the rB_RIF^S-specific sPDz probe as a function of rB_RIF^S concentration. **B:** Response of the rB_T_RIF^R-specific sPDz probe as a function of rB_T_RIF^R concentration. **C:** Response of the rB_G_RIF^R-specific sPDz probe as a function of rB_G_RIF^R concentration. **Panels A-C:** The signal is plotted as $\Delta A/A_0$, where $\Delta A = A - A_0$, and A_0 and A are absorbance values for the samples in the absence of presence of the target, respectively. *Inset:* linear dependence of the signal on the target concentration in the range of 0-20 nM (**A** and **B**) or 0-10 nM (**C**) and the limit of detection. **D:** Visually observed signal triggered by correspondent specific target at the indicated concentrations. The samples in the tubes (top-down) correspond to those used for **panels A-C**.

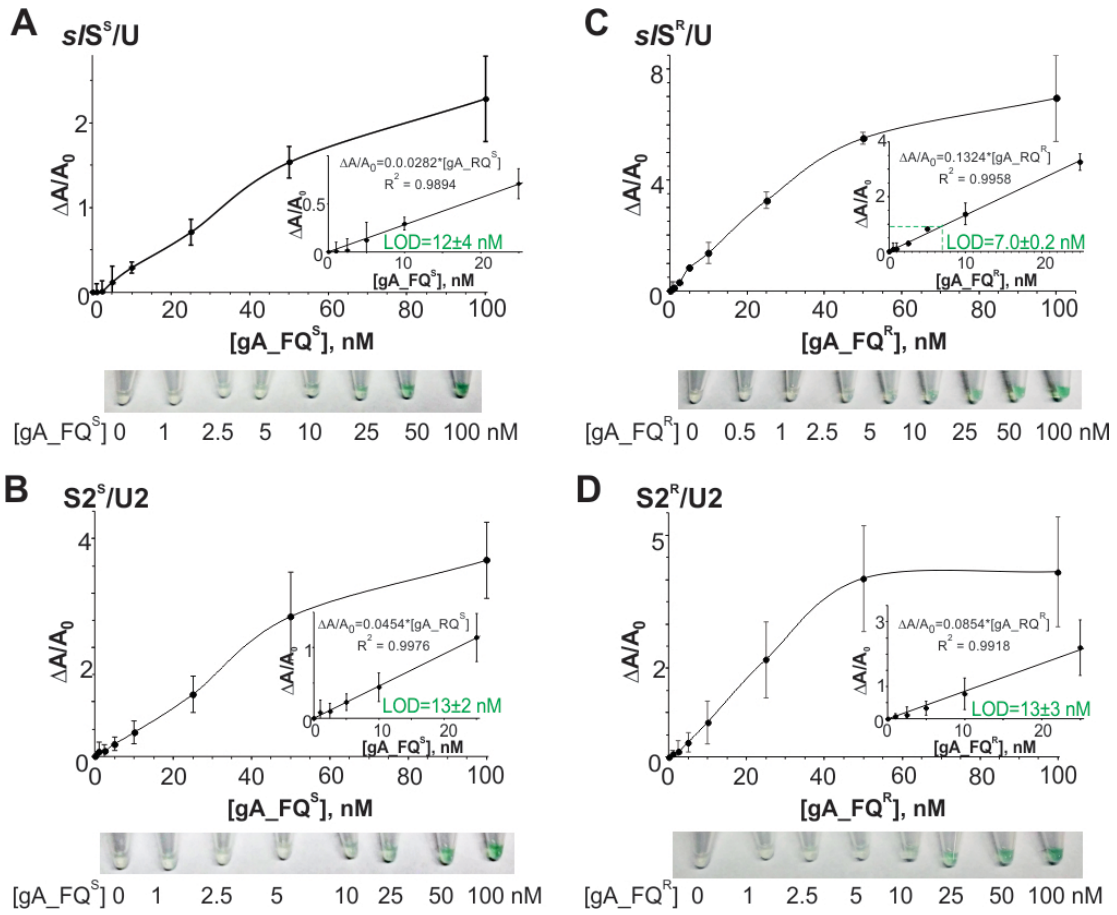

Figure S8. Performance of the sDz/PDz probes interrogating a fragment of the *gyrA* gene. A. Response of the probe consisting of U_gA and *sl*S_gA_FQ^S strands as a function of gA_FQ^S concentration. **B.** Response of the probe consisting of U2_gA and S2_gA_FQ^S strands as a function of gA_FQ^S concentration. **C.** Response of the sPDz probe consisting of U_gA and *sl*S_gA_FQ^R strands as a function of gA_FQ^R concentration. **D.** Response of the sPDz probe consisting of U2_gA and S2_gA_FQ^R strands as a function of gA_FQ^R concentration. The signal is plotted as $\Delta A/A_0$, where $\Delta A=A-A_0$, and A_0 and A are absorbance values for the samples in the absence of presence of the target, respectively. *Inset:* linear dependence of the signal on the target concentration in the range of 0-25 nM and the limit of detection. *Bottom:* Images of the tubes for the samples used for the plots.

Table S2. Sequences of the primers for NASBA used in the study.

Gene amplified	Forward primer[a]	Reverse primer	Size of the amplicon, nt[b]
rrs	AATTCTAATACGACTCACTATAGGGAGAAGGAG CCTGGGAAACTGGGTCTAA	GGCTCATCCCACACCGCTAA	95
katG	AATTCTAATACGACTCACTATAGGGAGAAGGG CTTGGGCTGGAAGAGCTCGTA	CCACTCGTAGCCGTACAGGAT	143

[a]Nucleotides containing the T7 RNA polymerase promotor sequence that are not complementary to the amplified gene are underlined. Nucleotides of the forward primer that are not transcribed to form the amplicon are in grey.

[b]Amplicons include a 9-nt fragment not corresponding to the gene sequence due to the additional 9 nt downstream the T7 RNA polymerase promotor.

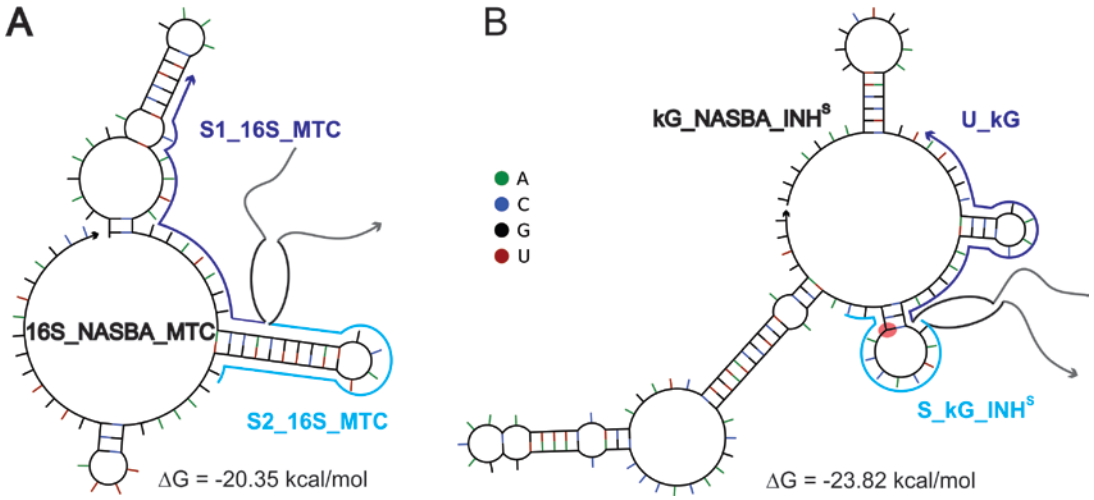

Figure S9. Minimum energy secondary structures for the targets 16S_NASBA_MTC (A) and kG_NASBA_INH^S (B) predicted by NUPACK (http://www.nupack.org). In the structures, nucleotides are color coded according to their nature (A, C, T or G, see color-coding in the middle). The target-recognition elements of the probe strands are schematically shown by blue and cyan curves, the subunits of the Dz catalytic core are shown as black semi-ovals, and the IPDz-binding fragments are represented by the grey curves. The 3'-termini of the targets of the probe strands are indicated by an arrow. In the structure of kG_NASBA, the position of the substituted nucleotide in the isoniazid-resistant genotype is highlighted by a red oval. The predicted values for the secondary structure free energy are indicated.

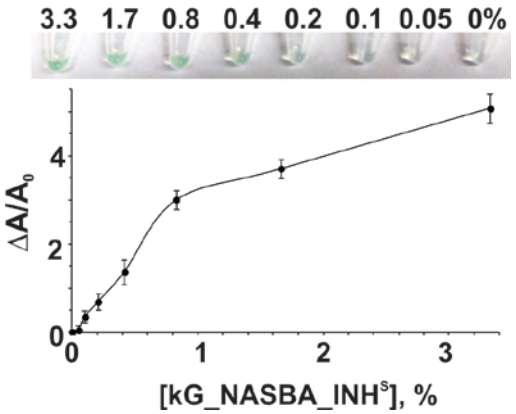

Figure S10. Response of the sDz/PDz probe targeting fragments of the *katG* gene to various amounts of the kG_NASBA_INH^S sample. The signal is plotted as $\Delta A/A_0$, where $\Delta A=A-A_0$, and A_0 and A are absorbance values for the samples in the absence of presence of the target, respectively. The NASBA sample was added to the assay sample without the amplicon's isolation at the indicated % (v/v). The data is average of three independent experiments, with the error bars presented as standard deviations. *Top:* Images of the tubes for the samples used for the plots.

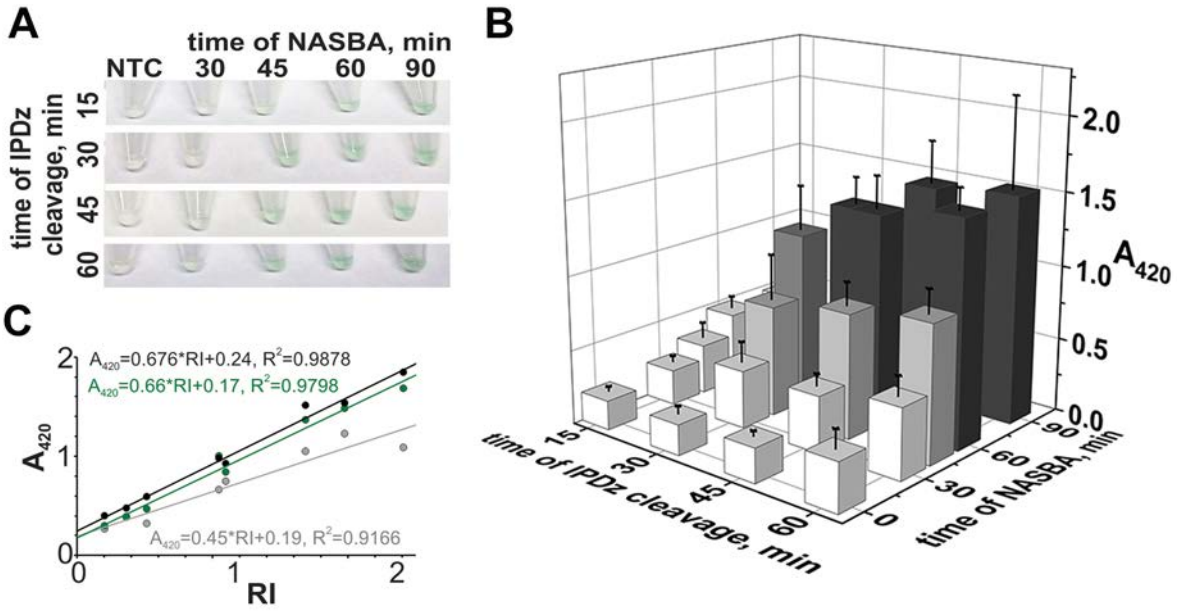

Figure S11. Time-dependence for NASBA/sDz stages of the sDz/PDz system interrogating a fragment of the *katG* gene. A and B. Analysis of the products of the NASBA reaction from panel A with total MTC RNA (83 ng/mL) in the colorimetric assay using the sDz/PDz system containing S_kG_INH^S and U_kG. The NASBA reaction was carried out for 30, 45, 60, or 90 min. The sample indicated as "0 min NASBA" is "no-target control" (NTC) for the NASBA reaction incubated for 90 min. The samples containing 3.3% NASBA samples were incubated at 50 ^{o}C for 15, 30, 45, or 60 min before visualized in the PDz-reaction**. A.** Images of the sample tubes. **B.** The data are average values for the absorbance at 420 nm (A_{420}) from three independent trials, with standard deviations as error bars. **C.** Linear correlation between A_{420} and intensity (RI) of the band corresponding to the NASBA amplicon upon gel electrophoresis analysis (Fig. 3A) relative to the intensity of a 200-nt band of the low-range RNA ladder. The data for 30, 45, and 60-min IPDz cleavage step is indicated with black, green, and grey data points and trendlines, respectively.

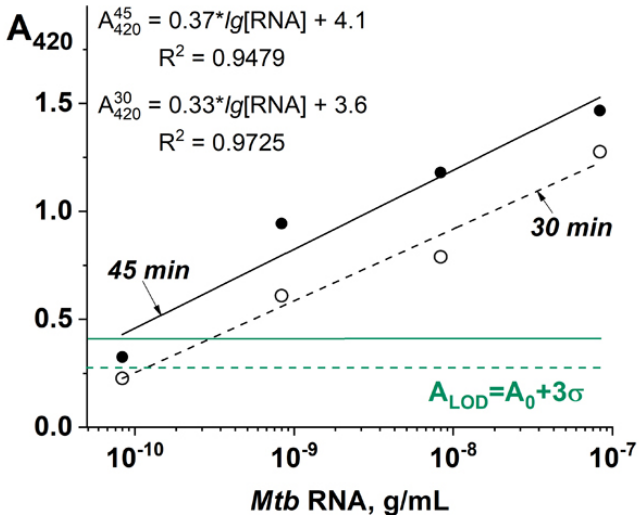

Figure S12. Dependence of the signal for the *katG*-specific NASBA/sDz system on the pre-amplified concentration of bacterial RNA. The samples containing the amplification products after 60-min NASBA (3.3%) were incubated at 50 ^{o}C for 30 min or 45 min. The data are average values from three independent trials. The trendlines were used to calculate the limit of detection (LOD) for the complete assay.