

Supplementary Material

Interrogation of Highly Structured RNA with Multicomponent Deoxyribozyme Probes at Ambient Temperatures

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Table S1. Oligonucleotides used in this work.

Name	Sequence ^a
tRNA ^{Phe}	GCG GAU UUA LCU CAG DDG GGA GAG CRC CAG ABU #AA YAP ?UG GAG 7UC ?UG UGT PCG "UC CAC AGA AUU CGC ACC A
DNA ^{Phe}	GCG GAC TTA GCT CAG TTG GGA GAG CGC CAG ACT GAA GAT CTG GAG GTC CTG TGT TCG ATC CAC AGA GTT CGC ACCA
DZ _{a55} ^{Phe}	<i>TGC CCA GGG AGG CTA GCT</i> cag gac ctc cag att ttc agt c
DZ _{b55} ^{Phe}	tgg tgc gaa ttc tgt gga tgc atc aAC AAC GAG AGG AAA CCT T
DZ _{a37.22} ^{Phe}	<i>CCA GGG AGG CTA GCT</i> cag gac ctc cag att ttc agt c 65°C
DZ _{a37.17} ^{Phe}	<i>CCA GGG AGG CTA GCT</i> cag gac ctc cag att tt 59.2°C
DZ _{a37.13} ^{Phe}	<i>CCA GGG AGG CTA GCT</i> cag gac ctc cag a 54.1°C
DZ _{a37.13C} ^{Phe}	<i>CCA GGG AGG CTA GCT</i> cag cac ctc cag a 55.6°C
DZ _{a37.11} ^{Phe}	<i>CCA GGG AGG CTA GCT</i> cag gac ctc ca 50.8°C
DZ _{b37.25} ^{Phe}	70.9°C tgg tgc gaa ttc tgt gga tgc aac aAC AAC GAG AGG AAA C
DZ _{b37.21} ^{Phe}	66°C gc gaa ttc tgt gga tgc aac aAC AAC GAG AGG AAA C
DZ _{b37.21A} ^{Phe}	66.4°C gc gaa ttc tgt gga acg aac aAC AAC GAG AGG AAA C
DZ _{b37.21C} ^{Phe}	67.6°C gc gaa ttc tgt gga ccg aac aAC AAC GAG AGG AAA C
DZ _{b37.21G} ^{Phe}	67.9°C gc gaa ttc tgt gga gcg aac aAC AAC GAG AGG AAA C
DZ _{b37.18} ^{Phe}	60.5°C aa ttc tgt gga tgc aac aAC AAC GAG AGG AAA C
DZ _{b37.13} ^{Phe}	53°C tgt gga tgc aac aAC AAC GAG AGG AAA C
MBP ^{Phe}	FAM – ATCGCT tetcaacctgagtaaat AGCGAT – Dabcyl
F _{sub}	AAG GT (FAM-T) TC CTC (rGrU) C CCT GGG CA-BHQ1
F _{sub} complement	TGC CCA GGG ACG AGG AAA CCT T
tRNA ^{Ser}	GGC ACU AUG GCM GAG D#G DDA AGG CRA CAG A<U NGA +AP CUG UJG GGC UCU GCC CG? GCU GGT PCA AAU CCU GCU GGU GUC GCC A
DNA ^{Ser}	GGC ACT ATG GCC GAG TGG TTA AGG CGA CAG ACT TGA AAT CTG TTG GGC TCT GCC CGC GCT GGT TCA AAT CCT GCT GGT GTC GCC A
DZ _{a37} ^{Ser}	<i>CCA GGG AGG CTA GCT</i> agc gcg ggc aga gcc caa cag a 75.5°C
DZ _{b37} ^{Ser}	72.5°C tgg cga cac cag cag gat ttg aac cAC AAC GAG AGG AAA C
DNA ^{18S}	<i>TATCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCA</i>
DZ _{a37} ^{18S}	<i>CCA GGG AGG CTA GCT</i> aag cat atg act act ggc a 62.4°C
DZ _{b37} ^{18S}	58.5°C tgg ctt aat ctt tga gac AC AAC GAG AGG AAA C

^a All sequences are written 5' to 3'. Symbols for modified bases (indicated in Table S2) are the same as those used at <http://trna.bioinf.uni-leipzig.de>. F_{sub}-binding regions of the probe strands are in italics. Nucleotide constituting the Dz core are in bold. Target-binding regions of the probe strands are shown with lower case letters. FAM-T is fluorescein attached to position 5 of the thymine ring by a 6-carbon spacer arm. BHQ1 is Black Hole Quencher®-1. The T_m values predicted by OligoAnalyzer (IDT) for the target binding fragments of the sDz strands in complex with their complements are indicated next to each strand sequence.

Table S2. Modified bases of the tRNA targets and the correspondent canonical bases used for the sequences of DNA analogs of the tRNA targets.

Modified base name	Symbol	Scheme 3 symbol	Base used for DNA analog
2'-O-methylcytidine	B	C _m	C
2'-O-methylguanosine	#	G _m	G
Wybosine	Y	yW	G
Pseudouridine	P	Ψ	T
5-methylcytidine	?	m ⁵ C	C
7-methylguanosine	7	m ⁷ A	G
1-methyladenosine	“	m ¹ A	A
dihydrouridine	D	D	T
N2,N2-dimethylguanosine	R	m ₂ ² G	G
N2-methylguanosine	L	m ² G	G
N4-acetylcytidine	M	N/A	C
unknown modified cytidine	<	N/A	C
unknown modified uridine	N	N/A	T
2'-O-methyluridine	J	N/A	T

SI-1. Effect of the length of F_{sub}-binding fragments of the sDz^{Phe} probes

In this work, we compared the performance of yeast tRNA^{Phe}-specific sDz probes differing in the length of the fragments interacting with the fluorogenic substrate (F_{sub}). The **sDz55^{Phe}** probe consisted of strands Dz_{a55}^{Phe} and Dz_{a55'}^{Phe} (Table S1) with the length of the F_{sub}-binding fragments optimized for 55°C. This resulted in high fluorescence of the samples in the absence of the target, and almost not increase of fluorescence upon addition of increasing target concentrations (Figure S1A). This can be attributed to the catalytic complex formation even in the absence of the target due to high affinity of the F_{sub}-binding fragments of Dz_{a55}^{Phe} and Dz_{a55'}^{Phe} (predicted T_m values were 51 and 43 °C, respectively) at the operating temperature of 37°C. As expected, the background increased with the sDz strand concentration (Figure S1B). No target-induced increase in signal could be attributed to the lack of catalytic turnover, since the products of F_{sub} cleavage would remain associated with the probe, thus preventing binding to new F_{sub} molecules. An alternative explanation of high background by mere stabilization of the extended conformation of the intact F_{sub}, in which the fluorophore is distant from the quencher, can be ruled out, at least for high strand concentrations, on the grounds of minimal increase in the substrate's fluorescence in the presence of the excess of F_{sub} complement (Figure S1C).

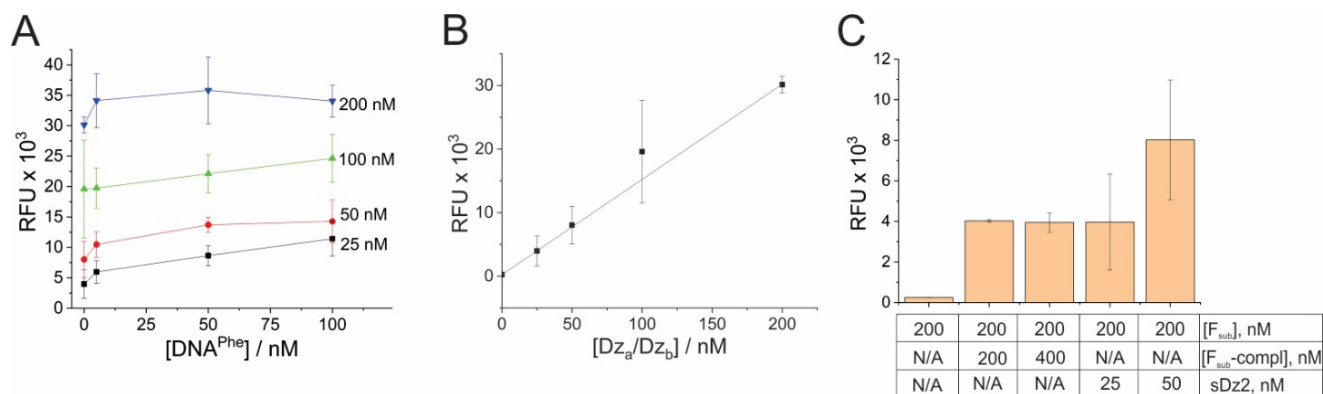


Figure S1. Performance of sDz55^{Phe}. **A.** Response of the probe (at different concentrations of Dz_{a55}^{Phe} and Dz_{b55}^{Phe}) as a function of DNA^{Phe} concentration (0-100 nM). **B.** Background of the probe (no target added) as a function of concentrations of Dz_{a55}^{Phe} and Dz_{b55}^{Phe} with linear regression analysis. **C.** Intensity of F_{sub} fluorescence in response to its complement or Dz_a/Dz_b. The concentrations of different sample components are indicated in the table below the bar graph. The data of three independent experiments was averaged, and the standard deviation was used to make the error bars.

The probe sDz37^{Phe} interrogated the same region of yeast tRNA^{Phe} as sDz55^{Phe} but had shorter F_{sub}-binding fragments. This allowed for the reduced background, as well as for increasing of target-dependent signal with the target concentration for all strand concentrations tested (Figure S2). High strand concentration helps shifting the equilibrium toward the catalytically active sDz-target complex. Therefore, we chose to use 200 nM Dz_a and Dz_b in this work.

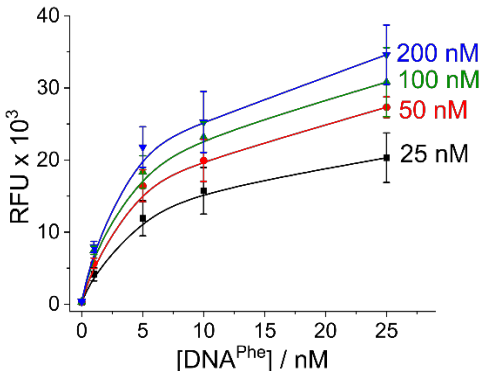


Figure S2. Performance of sDz37^{Phe}. The probe sDz37^{Phe} containing strands Dz_{a37.22}^{Phe} and Dz_{b37.25}^{Phe} at the indicated strand concentrations was incubated in the presence of increasing concentrations (0-25 nM) of the DNA^{Phe} target. Fluorescence intensity of the samples was plotted as a function of DNA^{Phe} concentration. The data of three independent experiments was averaged, and the standard deviation was used to make the error bars.

SI-2. Effect of the length of the target-binding fragments of sDz37^{Phe}

To find the minimal probe length needed for target detection, we analyzed a series of sDz37^{Phe} probes sharing the same F_{sub}-binding fragments and the catalytic core sequence but varied in the T_m of their target-binding regions. As can be seen in Figure S3A, a T_m for each of the target-binding regions of at least ~60°C was required for the detection of 25 nM tRNA^{Phe} target, using an S/B detection threshold of 3. A similar analysis was performed with DNA^{Phe} (Figure S3B), which showed that the target binding regions with lower T_m can be used for interrogating the DNA target.

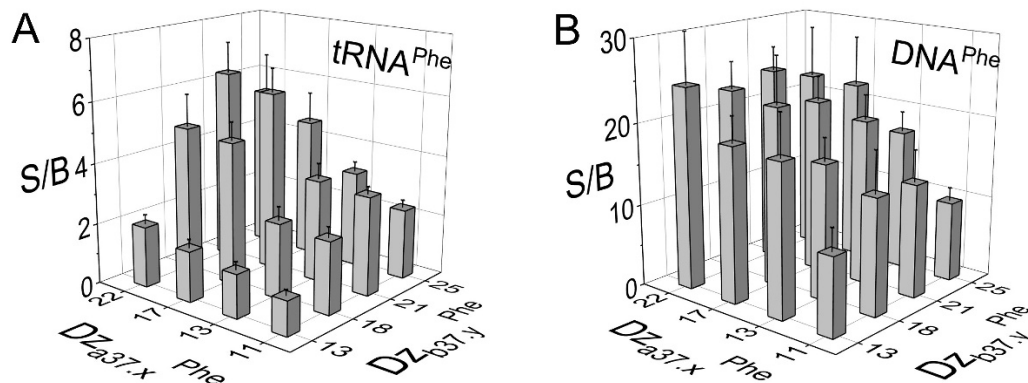


Figure S3. Response of the sDz37^{Phe} probes differing in the length of the target-binding fragments to yeast tRNA^{Phe} (A) or its DNA counterpart (DNA^{Phe}) (B). The sDz37^{Phe} probes differing in the length of their target-binding fragments and, correspondingly, in the affinity to their complementary target, were incubated in the absence or presence of the target (25 nM) for 70 min at 37 °C. The response is expressed as signal-to-background ratio (S/B). Data of three independent experiments with standard deviations as error bars are shown.

SI-3. Kinetics of F_{sub} cleavage by sDz37^{Phe} in the presence of tRNA^{Phe} or DNA^{Phe} target

Kinetics of F_{sub} cleavage by sDz37^{Phe} in the presence of different concentrations of tRNA^{Phe} or DNA^{Phe} was studied using the probe consisting of Dz_{a37.22}^{Phe} and Dz_{b37.21}^{Phe} (Table S1). The background was measured in the absence of the targets. No increase of the background signal was observed over time (within 5 h) (Figure S4A). Under the single turnover conditions (at 200 nM DNA^{Phe}), F_{sub} was completely cleaved within the first 10-20 min. For lower concentration of the DNA target, prolonged incubation for the substrate with the probe was required for the quantitative F_{sub} cleavage.

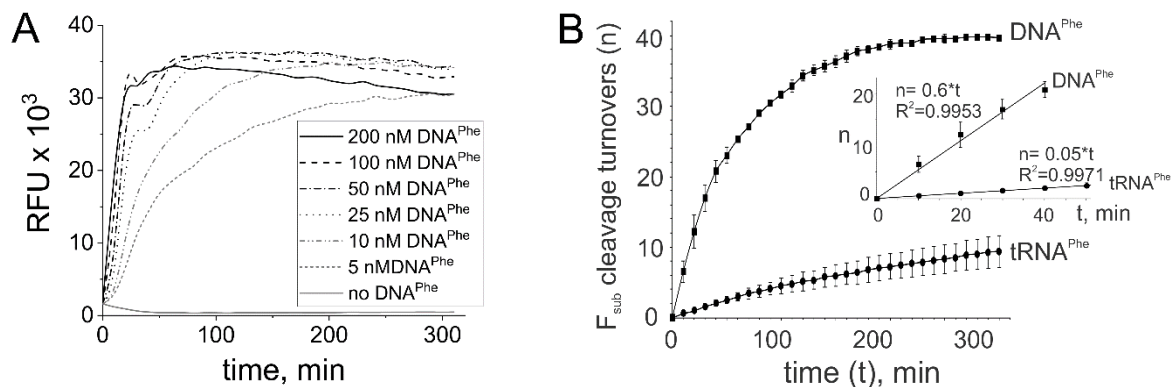


Figure S4. Kinetics of F_{sub} cleavage by sDz37^{Phe} at different target concentrations. **A.** The signal exhibited by the probe containing strands Dz_{a37.22}^{Phe} and Dz_{b37.21}^{Phe} in the presence of varying concentrations of DNA^{Phe} (5-200 nM) was measured over time (every 10 min within 5 h). **B.** Plot of the number of turnovers (n) of F_{sub} cleavage under multiple turnover conditions (at 5 nM tRNA^{Phe} or DNA^{Phe}, as indicated) over time. *Inset:* Linear dependence of the number of turnovers (n) on time within first 40-60 min. The slope of the linear trendline represents the observed rate constant (k_{obs}) for F_{sub} cleavage.

The data was used to determine the number of turnovers for the substrate cleavage catalyzed by the deoxyribozyme core formed upon interaction of sDz with tRNA^{Phe} or DNA^{Phe} target at

different concentration (5-200 nM) (Table S3). For this purpose, the percent and concentration of cleaved F_{sub} was calculated by dividing the fluorescence intensity of the target-containing sample after subtraction of the background (no-target sample) at each of the indicated target concentrations by the maximal intensity for the sample containing 200 nM target (single turnover conditions). To calculate the number of cleavage rounds, it was assumed that each target molecule triggers formation of the catalytic core by interacting with Dz_a and Dz_b . The calculated concentration of the cleaved F_{sub} for each target concentration was divided by the target concentration to obtain the number of cleavage rounds (turnovers).

The observed rate constant for F_{sub} cleavage (k_{obs}) was calculated as a slope of a linear trendline obtained by plotting the number of cleavage rounds (turnovers) for the sample containing 5 nM target (multiple turnover condition) (Figure S4B) as a function of time for the first 60 min or 40 min for $tRNA^{\text{Phe}}$ and DNA^{Phe} targets, respectively. About 10-fold difference in the rate constant was observed for the same concentration of $tRNA^{\text{Phe}}$ and DNA^{Phe} targets ($k_{\text{obs}}=0.05\pm 0.01 \text{ min}^{-1}$ and $0.6\pm 0.1 \text{ min}^{-1}$, respectively).

Table S3. Catalytic turnover of $sDz37^{\text{Phe}}$ triggered by $tRNA^{\text{Phe}}$ and DNA^{Phe}

[Target], nM	Number of F_{sub} cleavage reactions at 37°C in 70 min ^a	
	$tRNA^{\text{Phe}}$	DNA
5	3.3±0.8	26±2
10	3±1	16.1±0.2
25	2.4±0.7	7.62±0.06
50	1.9±0.4	3.94±0.05
100	1.3±0.2	2.00±0.01
200	0.82±0.03	1.00±0.01

^aThe $sDz37^{\text{Phe}}$ probe contained strands $Dz_{a37.22}^{\text{Phe}}$ and $Dz_{b37.21}^{\text{Phe}}$. The data of three experiments were analyzed independently, and the calculated numbers were averaged with an error in the form of one standard deviation.

SI-4. Limit of detection of $tRNA^{\text{Phe}}$ and DNA^{Phe} target by $sDz37^{\text{Phe}}$ or MBP^{Phe}

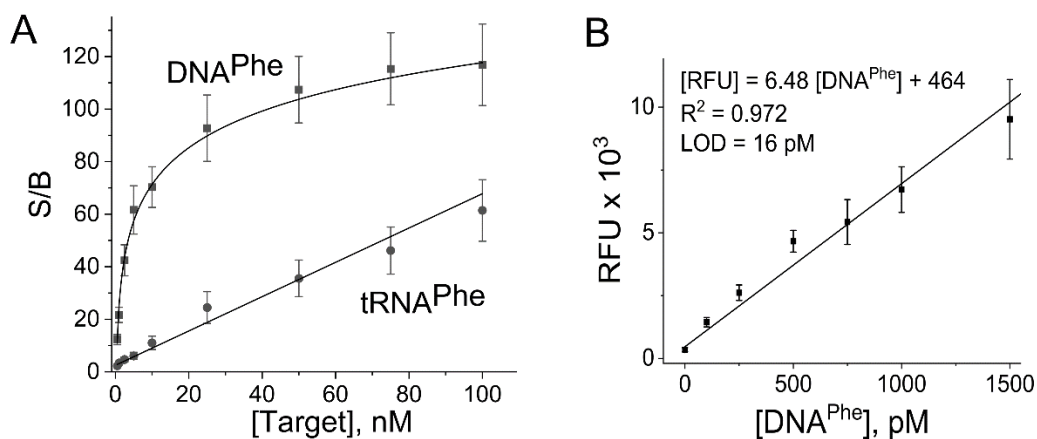


Figure S5. Dependence of $sDz37^{\text{Phe}}$ signal on the target concentration. A. Signal-to-background ratio (S/B) for the probe containing strands $Dz_{a37.22}^{\text{Phe}}$ and $Dz_{b37.21}^{\text{Phe}}$ at varying concentrations (0-100 nM) of DNA^{Phe} (squares) or $tRNA^{\text{Phe}}$ (circles) with the logarithmic or linear fit, respectively. **B.** Limit of

detection (LOD) of DNA^{Phe} by sDz37^{Phe}. LOD was determined by dividing three standard deviations of the average blank (no-target background) by the slope of the best fit line.

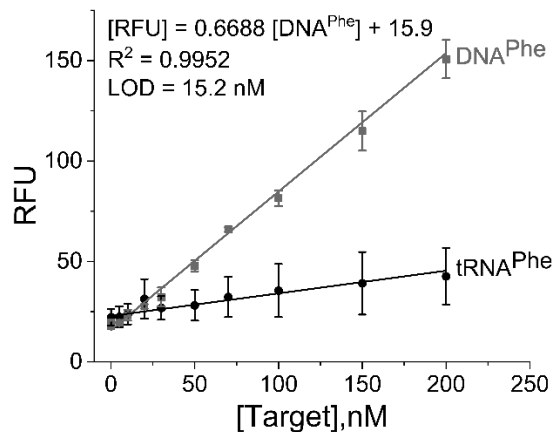


Figure S6. Interrogation of tRNA^{Phe} or DNA^{Phe} by MBP^{Phe}. Target-dependent increase in the signal of the MBP^{Phe} probe targeting the D-arm of the tRNA^{Phe}. Response of the MBP^{Phe} probe to tRNA^{Phe} or DNA^{Phe} is shown by black and grey datasets, respectively. For both targets, the MBP was used at 50 nM. Limit of detection (LOD) was determined by dividing three standard deviations of the average blank (no-target background) by the slope of the best fit line.

SI-5. sDz probes interrogating other RNA target

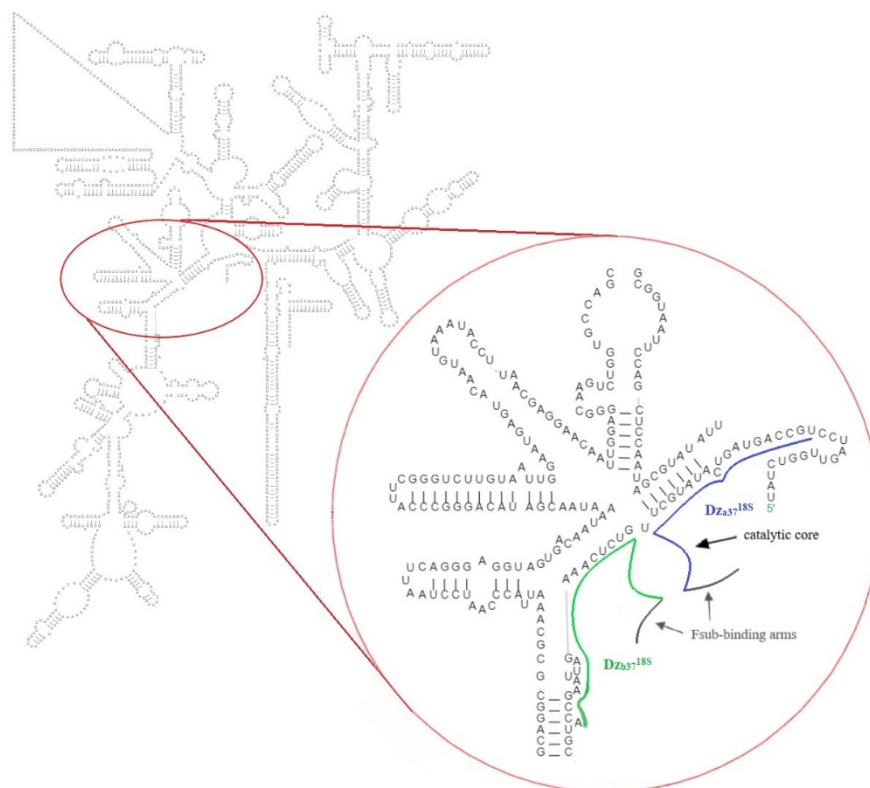


Figure S7. Structure of *S. cerevisiae* 18S rRNA interrogated by sDz37^{18S}. Secondary structure of yeast 18S rRNA from RNA Central (<https://rnacentral.org/rna/URS00005F2C2D/559292>) with the enlarged fragment showing interrogation by Dz_{a37}^{18S} and Dz_{b37}^{18S} (Table S1).

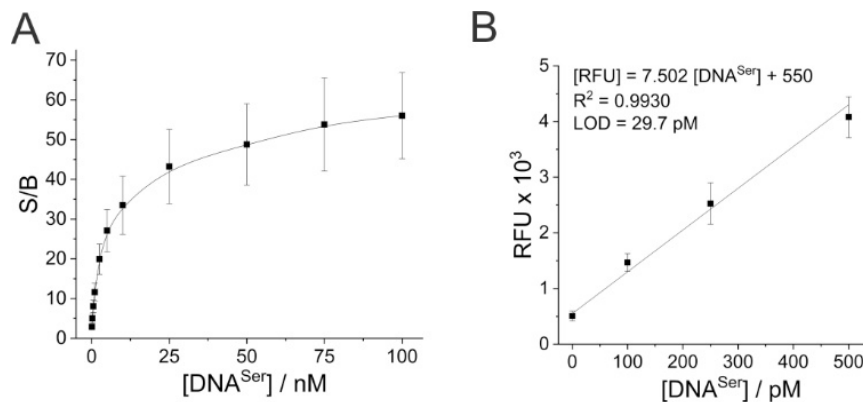


Figure S8. Response of sDz37^{Ser} to varying concentrations of DNA^{Ser}. A. 0-100 nM DNA^{Ser}. B. 0-500 pM DNA^{Ser}.

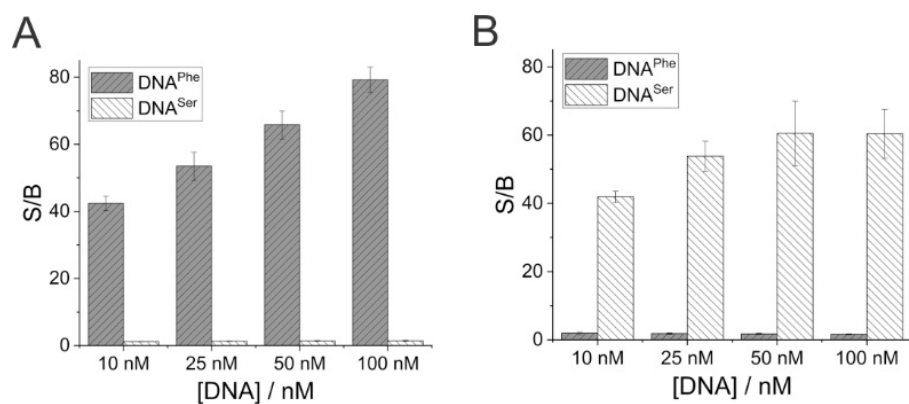


Figure S9. Selectivity of sDz37^{Phe} (A) and sDz37^{Ser} (B) demonstrated with synthetic DNA analogs of the tRNA targets: DNA^{Phe} and DNA^{Ser}. Each probe was incubated with its specific or non-specific synthetic DNA target (Table S1) at the indicated concentrations.

SI-6. sDz probes for species-specific cell growth monitoring

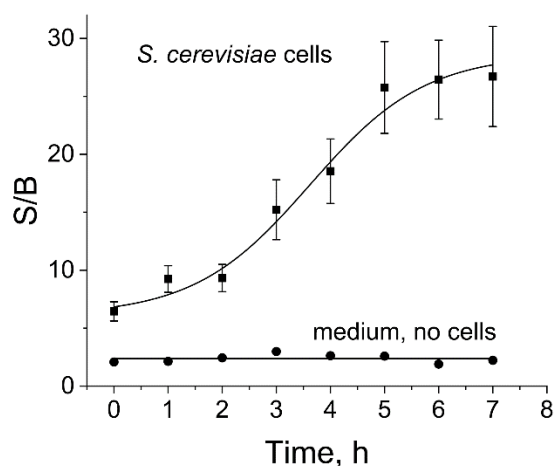


Figure S10. *S. cerevisiae* cell grow curve as monitored via sDz37^{Phe} response. The data of three trials is averaged and fitted to a sigmoidal curve. The response of the probe to the same liquid medium in the absence of the yeast cells is also shown.

Supplementary references

1. Santoro, S. W. & Joyce, G. F. A general purpose RNA-cleaving DNA enzyme. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 4262–4266 (1997).
2. Shizuka, H., Nakamura, M. & Morita, T. Anion-induced fluorescence quenching of aromatic molecules. *J. Phys. Chem.* **84**, 989–994 (1980).