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Supplementary Material

DNA Probes for Cas12a-Based Assay with Fluorescence Anisotropy Enhanced due to Anchors and Salts

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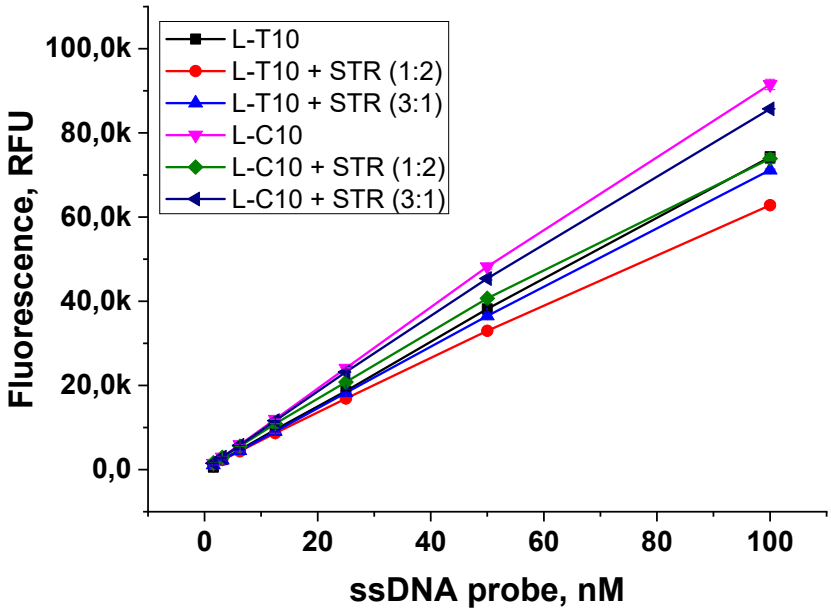


Figure S1. Dependences of fluorescence on ssDNA concentration in the absence of streptavidin (STR), as well as at molar ratios of probe : STR of 1:2 and 3:1. Experiments were carried out in 20mM Tris-HCl buffer, pH 9.

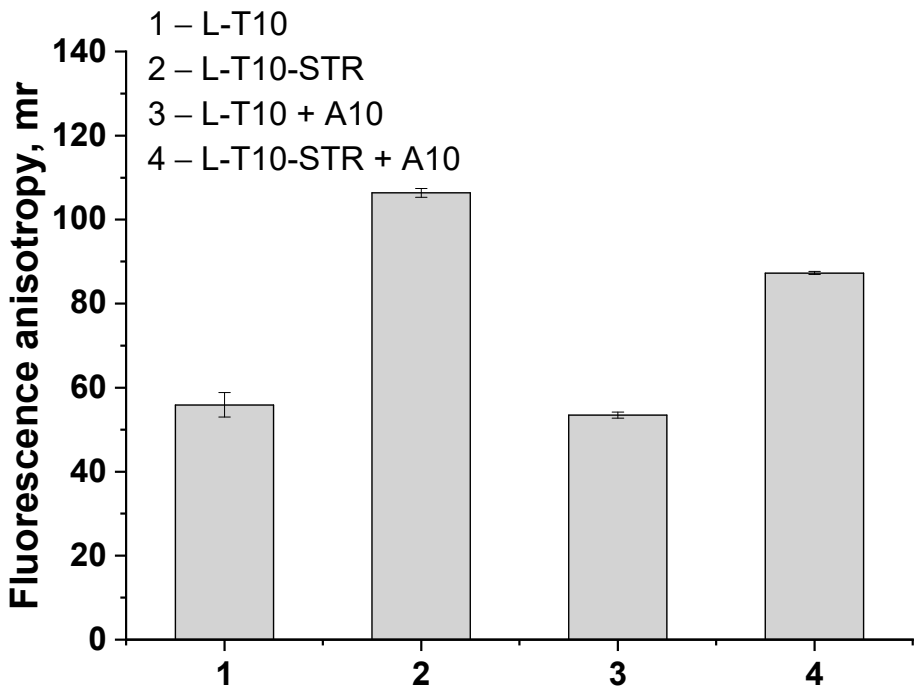


Figure S2. Comparison of the fluorescence anisotropy of probe L-T10 and STR – L-T10 before and after interaction with A10. Experiments were carried out in 20 mM Tris-HCl buffer containing 10 mM Mg^{2+}, pH 9.

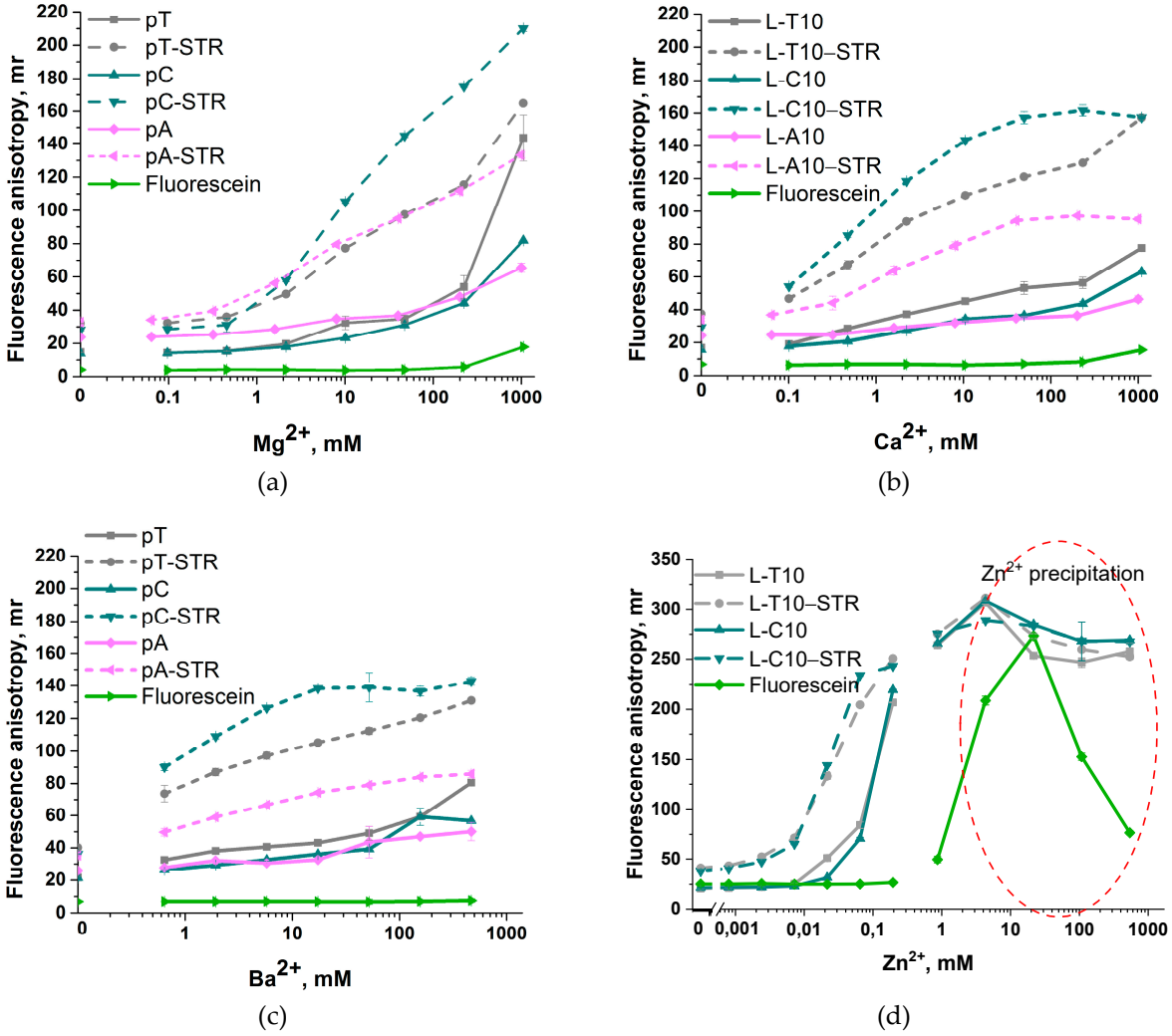


Figure S3. Fluorescence anisotropy of L-T10, L-C10, L-A10 and their conjugates with streptavidin anchor at 1:2 molar ratio depending on the concentration of Mg^{2+} (a), Ca^{2+} (b), Ba^{2+} (c), and Zn^{2+} (d) in 50mM Tris-HCl, pH 9.

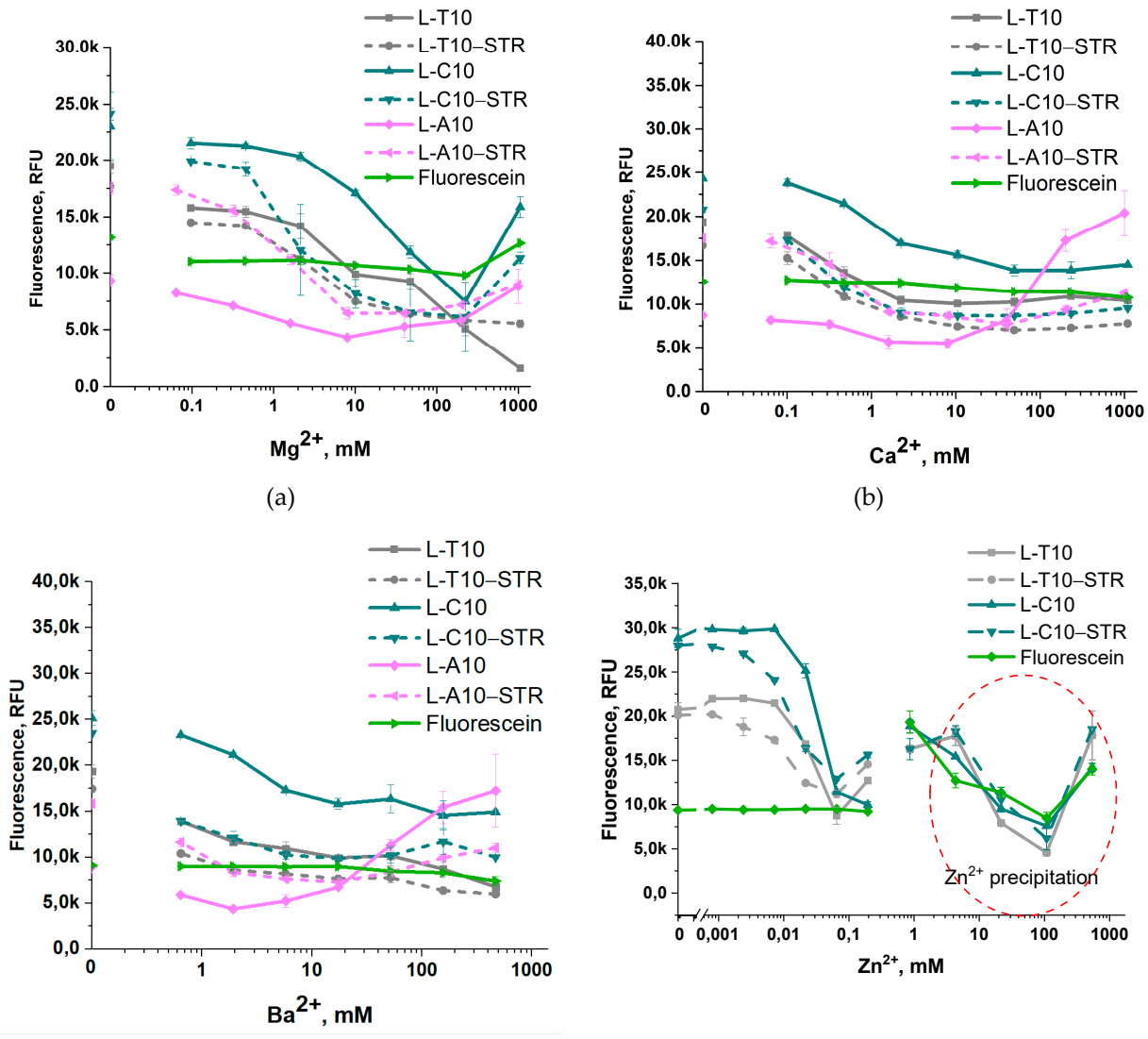
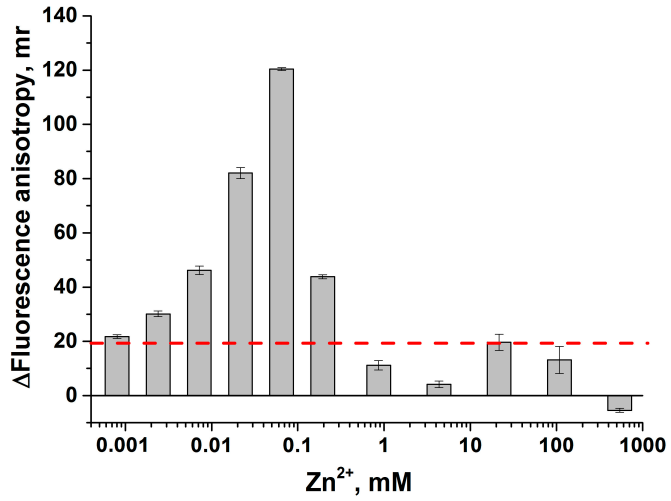
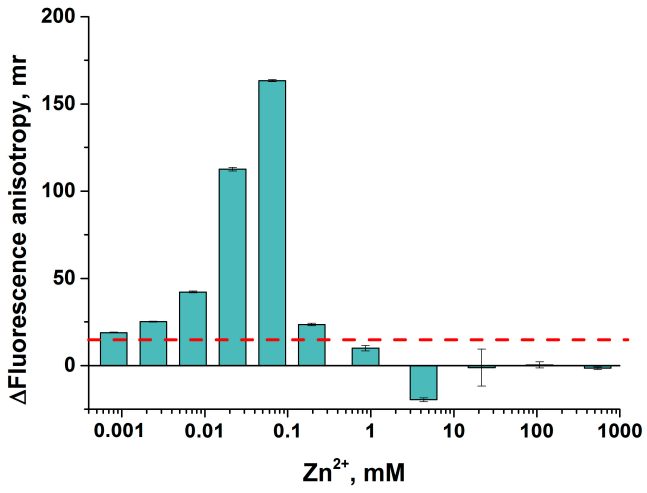


Figure S4. Fluorescence of L-T10, L-C10, L-A10 and their conjugates with streptavidin anchor at 1:2 molar ratio depending on the concentration of Mg^{2+} (a), Ca^{2+} (b), Ba^{2+} (c), and Zn^{2+} (d) in 50mM Tris-HCl, pH 9.

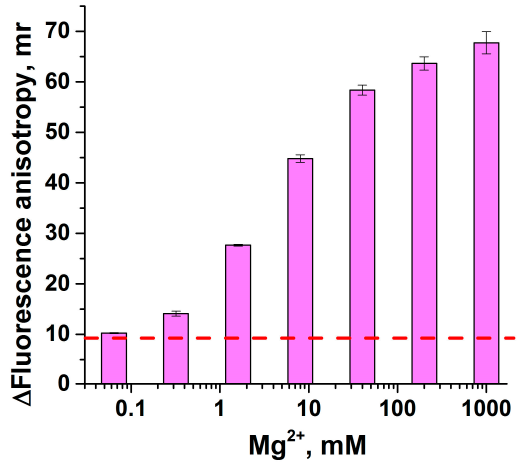


a

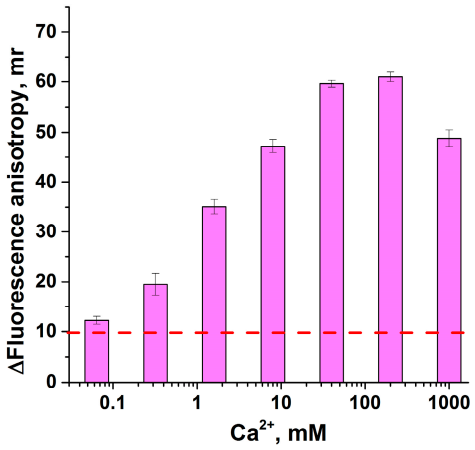


b.

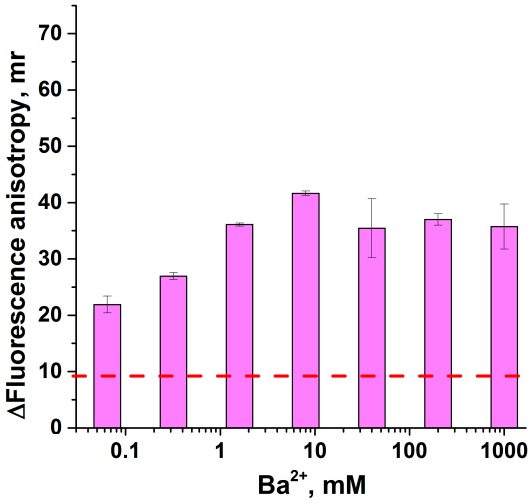
Figure S5. Fluorescence anisotropy difference between STR-probe and probe at different concentrations of Zn^{2+}. (a) For L-T10 probe, (b) for L-C10 probe. The dashed line indicates ΔFA in the absence of Zn^{2+}.



(a)



(b)



(c)

Figure S6. Impact of divalent metal ions on fluorescence anisotropy (FA) of L-A10 probe and probe with STR. The difference in FA of SRT-probe and probe on the concentration of Mg^{2+} (**a**), Ca^{2+} (**b**), and Ba^{2+} (**c**). The dashed lines indicate ΔFA in the absence of Me^{2+}.

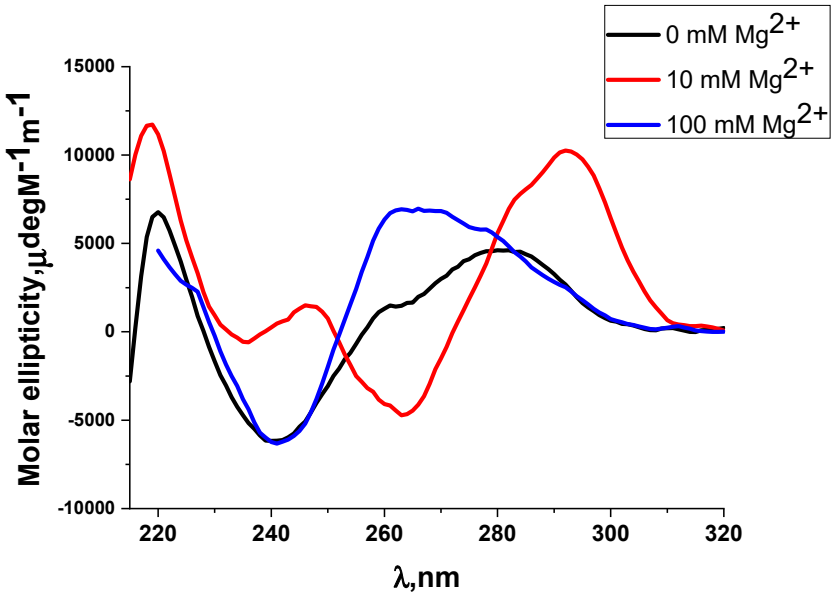


Figure S7. CD spectra of G-quadruplex carried out in 50mM Tris-HCl buffer, pH 9 at different concentrations of $Mg(CH_3COO)_2$. The reagent concentrations are 1 μM for all spectra.

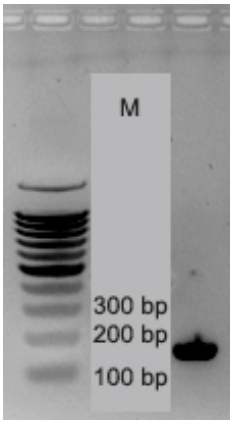


Figure S8. Scan of gel electrophoresis in 2% agarose gel of fragment of target AMY1267 gene of *Erwinia amylovora*. M – dsDNA ladder.

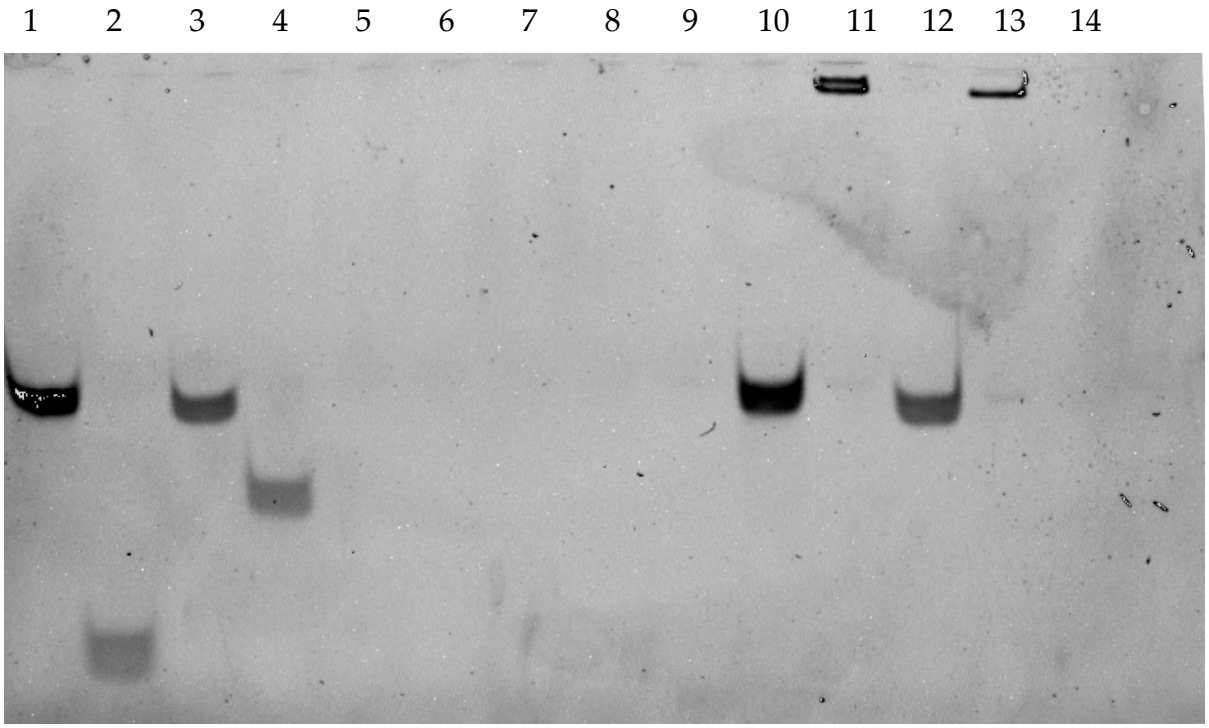


Figure S9. Scan of polyacrylamide gel after electrophoresis of ssDNA probes (1 – G-quadruplex, 2 – L -C10, 3 – H-R21, 4 – H-C10), mixtures after Cas12a-based assay with 10 nM dsDNA and ssDNA probes (5 – G-quadruplex, 6 – L -C10 + STR after assay, 7 – H-R21, 8 – H-C10 + STR before assay, 9 – without probe), mixtures after Cas12a-based assay without dsDNA and with ssDNA probes (10 – G-quadruplex, 11 – L -C10 + STR after assay, 12 – H-R21, 13 – H-C10 + STR before assay, 14 – without probe).

Table S1. Sequences of primers and guide RNA used in this research.

Name	Sequence 5′-3′	Purpose
F-PCR	CCGTGGAGACCGATCTTTTA	Forward primer for PCR
R-PCR	AAGTTTCTCCGCCCTACGAT	Reverse primer for PCR
F-RPA	GCTCTCATTGCCGTGGAGACCGATCTTTTA	Forward primer for RPA
R-RPA	TTATAACAAAAGTTTCTCCGCCCTACGAT	Reverse primer for RPA primer R
gRNA	UAAUUUCUACUAAGUGUAGAUAGAGAG GCAGCAUUCGACGAAC	gRNA for *E. amylovora* recognition