DOI 10.18699/SBB-2022-200

# DNA-nanomachines for nucleic acid detection

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**Key words:** DNA-nanomachines, DNAzyme, G-quadruplex, nucleic acid detection

*Motivation and Aim*: A DNA-nanomachine (DNM) is a powerful tool for nucleic acid detection. DNMs are oligonucleotide-based multifunctional nanostructures that can tightly bind nucleic acids, including folded single stranded (ss) RNA or double stranded (ds) DNA targets. DNMs can recognize single nucleotide substitutions (SNS) with a high selectivity and provide a readable output. DNMs are compatible with point-of-care (POC) diagnostics. We report here two DNM variants: (i) DNAzyme-based DNM with fluorescent output [1] (Fig. 1) and (ii) G-quadruplex based DNM with visual output [2] (Fig. 2).

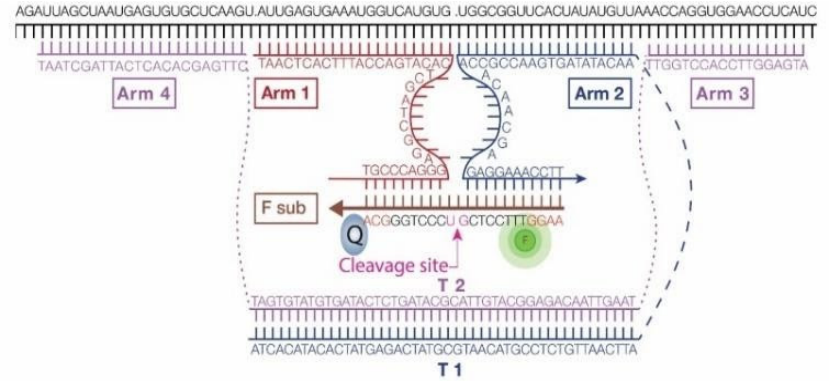


**Fig. 1.** An example of a DNA-nanomachine for fluorescence detection of coronavirus

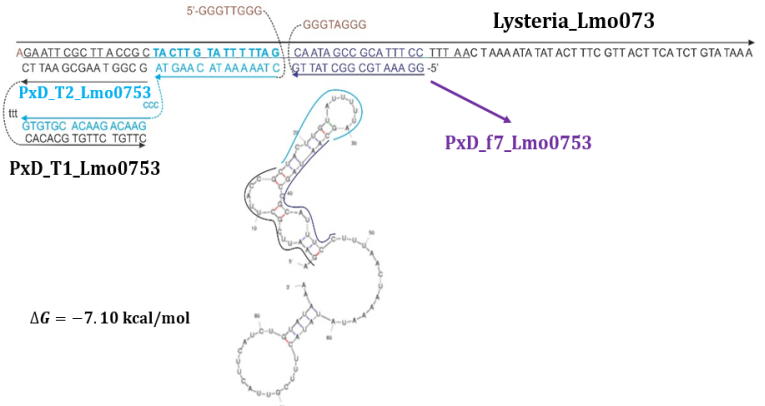


**Fig. 2.** An example of a DNA-nanomachine for colorimetric detection of Lysteria monocytogenes and the fragment of interest

*Methods and Algorithms*: A series of DNM targeting sequence of 20 human pathogens were designed. We took in account predicted RNA secondary structure and optimized DNMs for differentiating SNS using UNAfold and NUPACK software. DNM were assembled from custom-made oligonucleotides and optimized for fluorescent or visual detection of synthetic ssDNA, dsDNA and RNA amplicons, unamplified bacterial rRNA or viral RNA of human pathogens.
*Results*: Optimized DNMs produced signals 1,5 to 4 above the background detect in all cases after 15 to 180 min assays. The sensitivity assays were down to 800 fM and in a micromolar range for fluorescent and visual outputs respectively. The sensitivity is dictated by the temperature of the environment, length of the recognition arms, its number, type of a core, and type of the nucleic acid. DNMs selectively recognized SNS under experimental conditions, including room temperature. The G-quadruplex-based DNM are suitable for rapid visual room temperature detection of dsDNA and RNA amplicons. The fluorescent DNMs can quantify nucleic acid without amplification, but require elevated temperature (55 ºC) and prolonged incubation time.
*Conclusion*: The results confirm that DNM can be adapted for detection of dsDNA and folded ssRNA of various sequences. The DNM can be adjusted for SNS differentiation under broad experimental conditions. They are made of unmodified synthetic DNA and, therefore, are relatively inexpensive. DNM can be incorporated in POC diagnostic tests for visual or fluorescent detection of human pathogens.
*Acknowledgements*: The study is supported by the RSCF grant (22-24-00664).

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