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**ALL-DNA SHORT OLIGONUCLEOTIDE STAPLE (SOS) PROBE FOR FLUORESCENT REPORTING OF DOUBLE STRANDED DNA ANALYTE AT LOW TEMPERATURES**M. Ateiah<sup>1,2</sup>, D. M. Kolpashchikov<sup>3,4</sup><sup>1</sup>Laboratory of DNA-Nanosensor Diagnostics, ITMO University, Saint Petersburg<sup>2</sup>Laboratory of Amyloid Biology, Saint Petersburg State University<sup>3</sup>Chemistry Department, University of Central Florida, Orlando, USA<sup>4</sup>Burnett School of Biomedical Sciences, University of Central Florida, Orlando, USA

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**Abstract**

Hybridization of probe to dsDNA is thermodynamically unfavorable and achieved by thermal denaturation of dsDNA or by using DNA analogs with increased affinity. We designed all-DNA probe that binds dsDNA analyte and produces fluorescent signal only at low temperatures. It can be designed to differentiate single base pair difference in dsDNA analyte with modest differentiation factor. The Limit of detection of the probe is 2 nM after 3h of incubation at 4 °C.

Probe hybridization to single-stranded DNA or RNA is thermodynamically favorable, as the formation of Watson-Crick base pairs stabilize the resulting complex [1]. However, the binding of a hybridization probe to double-stranded DNA (dsDNA) presents a significant thermodynamic challenge [2]. When a probe binds to dsDNA, the number of newly formed base pairs is less than or equal to the number of disrupted base pairs, as the unpaired fragment of the original duplex opposes the bound probe. Moreover, probe binding to dsDNA results in an unfavorable entropy change, as it reduces the configurational entropy of the system. Consequently,  $\Delta G^\circ$  for probe/dsDNA complex formation is typically positive, rendering spontaneous hybridization unfavorable under standard conditions.

Probe hybridization to dsDNA is typically achieved by thermal denaturation of dsDNA in the presence of excess amounts of a hybridization probe or by using DNA analogs with increased affinity to DNA including Peptide Nucleic Acid (PNA) and Locked Nucleic Acid (LNA) -based probes [3, 4]. Here, we present an alternative strategy for sequence-specific recognition of dsDNA under non-denaturing conditions. Our approach is based on a multicomponent sensor composed of short oligonucleotide staple (SOS) strands designed to invade dsDNA by thermodynamically favoring complex formation at low temperature.

$G = H - TS$ . The entropy factor has reduced contribution to the  $G$  of complex formation at reduced temperatures. Therefore, theoretically, the lower the temperature, the higher the probability of complex formation between SOS sensor and dsDNA. Unlike conventional approaches, the SOS sensor does not rely on denaturing conditions, chemical modification, or protein-based invasion. Instead, the binding is driven by the net gain in base pairing: upon hybridization, the cumulative number of base pairs formed between SOS and the target DNA exceeds the number of disrupted base pairs within the duplex. The SOS sensor forms 58 base pairs upon target invasion while displacing only 20 base pairs of the target duplex. This design enables dsDNA invasion and stable probe-dsDNA complex formation at 4 °C.

The SOS sensor is also designed to trigger fluorescence via a target-induced conformational change in a universal molecular beacon (UMB). The SOS comprises four strands; among them, two (SOS-A and SOS-B) are engineered with dual functionality: each contains a dsDNA-binding arm and a UMB-binding arm connected by a flexible hexaethylene glycol (HEG) linker. Upon successful invasion of the target duplex, the UMB-binding arms stabilize the open conformation of the beacon, disrupting the stem-loop structure and separating the fluorophore from the quencher. This conformational switch results in a fluorescence turn-on signal that directly correlates with invasion efficiency and is applicable to studies both at equilibrium and near-equilibrium (time-dependent) conditions. The SOS sensor achieved a signal-to-background (S/B) ratios of 2.6 and 5.1, after 1 and 3 h of incubation at 4 °C, respectively. The limit of detection was found to be 2 nM after 3 h of incubation.

This work presents a novel design concept for all-DNA SOS sensor capable of sequence-specific dsDNA recognition under non-denaturing conditions. By leveraging thermodynamically favorable binding at low temperatures, the sensor achieves stable duplex invasion and fluorescence-based detection without chemical modification or thermal denaturation. With a detection limit of 2 nM and the ability to differentiate single base pair mismatches, the SOS sensor offers a simple and effective platform for DNA diagnostics.

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**References**

1. Pierce K. E., Wang L. J. LATE-PCR and allied technologies: real-time detection strategies for rapid, reliable diagnosis from single cells // *Methods in Molecular Biology*. 2011. Vol. 688. P. 47–66.
2. Stancescu M., Fedotova T.A., Hooyberghs J. et al. Non-equilibrium hybridization enables discrimination of a point mutation within 5–40 °C // *Journal of the American Chemical Society*. 2016. Vol. 138. P. 13465–13468.
3. Muangkaew P., Vilaivan T. Modulation of DNA and RNA by PNA // *Bioorganic & Medicinal Chemistry Letters*. 2020. Vol. 30. P. 127064.
4. Veedu R. N., Wengel J. Locked nucleic acids: promising nucleic acid analogs for therapeutic applications // *Chemistry & Biodiversity*. 2010. Vol. 7. P. 536–542.