An Integrated ElectroSensor Based on Toehold-mediated Strand Displacement-induced Formation of DNA-immuno-superstructure

Amily Fang-Ju Jou, Wei-Ching Liao, and Ja-an Annie Ho*

Department of Biochemical Science and Technology
National Taiwan University, No 1, Sec. 4, Roosevelt Rd., Taipei Taiwan

DNA technology, via the specific recognition of the complementary base pairing, has led to the new era of sensing applications. The renowned programmable system previously demonstrated began with the toehold-mediated strand displacement (TSD) that utilized an enthalpy-driven opening/closing process in response to a target or substrate [1]. With rational sequence design, a serial of DNA-based nanomachines, DNA gears, and logic circuits, have emerged as mainstream nano-implements to achieve autonomous and programmable systems [2–4]. In particular, the DNA circuits have proven to be versatile functional units for integration. A toehold-integrated strand displacement reaction-triggered signal amplification cascade strategy is employed herein to engineer a versatile electrochemical operation for the detection of a single-nucleotide polymorphism (SNP) as a powerful and valid screening test for the detection of Alzheimer's disease. In addition to the incorporation of a toehold domain in a pre-hybridized DNA duplex, our design also includes an additional modification of a displacing strand, the trigger. This trigger, extended with an additional overhang of a few more nucleotides in a staggered position relative to the toehold domain, is utilized to kinetically control the displacement reaction and initiate a primary nicking enzyme amplification reaction. The generation of amplicon, as a result of the primary amplification reaction, inaugurates the formation of a superstructure upon the opening of the biotin-tagged hairpin and biotin/(streptavidin) recognition on the gold electrode. The DNA-immuno superstructure thereby activates an electrochemical response where a horseradish peroxidase (HRP)/TMB/H2O2 system or liposomal Ru(Hex) tags served as the signal output for the secondary signal amplification. We substantiated the autonomous operation of the strategy and successfully highlighted the application in the determination of a single nucleotide change as an indicator of risk for Alzheimer's disease.

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