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論文 / 著書情報
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著者(和文)	WANG Jun
Author(English)	Jun Wang
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（博士課程）
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論 文 要 旨

THESIS SUMMARY

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学生氏名： Student's Name	20D48128		審査員主査： Chief Examiner	Atsushi Maruyama	

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Dynamic DNA circuits based on toehold-mediated DNA strand displacement have been the most important approaches to achieve various functions in the field of dynamic DNA nanotechnology and nanoscience. DNA hybridization chain reaction (HCR) and DNA catalytic hairpin assembly (CHA) are two representative and practically important dynamic DNA circuits. HCR circuit proceeds through the continuous cross-hybridization of two metastable DNA hairpin reactants which is initiated by a single stranded initiator, resulting in generation of long DNA polymers. In CHA circuit, the cross-opening of two DNA hairpins in the presence of initiator produces numerous DNA duplexes. These DNA circuits have been widely applied in various applications such as biosensing, cancer theranostics, and execution of computing tasks due to their programmable self-assembly processes and nonenzymatic and isothermal amplification properties. However, their further progress and applications has been impeded by their limitations including low operation rates and low robustness. Therefore, a strategy to improve the performance of DNA circuits is highly desired.

The cationic copolymer, poly($_L$-lysine)-*graft*-dextran (PLL-*g*-Dex) reported by Maruyama group, forms soluble inter-polyelectrolyte with nucleic acids. The copolymer promotes DNA hybridization and enhances the thermostability of DNA duplex. In this study, the effects of PLL-*g*-Dex on three well-established DNA circuits: HCR, CHA, and a two-input AND logic circuit were investigated. The cationic copolymer significantly augments the performance of DNA circuits, accelerating the operation speed by more than 40-fold and improving their robustness against nuclease digestion, molecular noise, and reaction conditions including temperature, and salt concentration.

The HCR circuit was analyzed by gel characterization and real-time fluorescence measurement. The cationic copolymer promoted the generation of HCR products with high molecular weight by increasing reaction rates of both the initiation step and subsequent cross-opening of hairpins as the copolymer shields the electrostatic repulsion of DNA strands. Therefore, addition of PLL-*g*-Dex accelerated HCR circuit by 42-fold, shortening the reaction time from 4 h to 10 min. The initiator sensitivity of HCR was improved from 3.3 nM in the absence of copolymer to 80 pM in the presence of copolymer. The copolymer also successfully promoted the HCR circuit initiated by microRNA let7a, indicating that PLL-*g*-Dex commonly accelerated different HCR circuits initiated by either DNA or RNA. The results of temperature dependence revealed that the copolymer enhances the effectiveness of HCR circuit in a wider temperature range under physiological salt condition. Furthermore, PLL-*g*-Dex protected HCR components from nucleases digestion, permitting the operation of circuit in a serum-containing medium.

The CHA circuit was employed to examine the general effectiveness of cationic copolymer for accelerating DNA circuit. The fluorescence assay showed that PLL-*g*-Dex promoted CHA circuit by 44-fold without causing significant circuit leakage which is the undesired reaction in the absence of initiator. Moreover, PLL-*g*-Dex greatly alleviated the dependency of CHA circuit on toehold length. In the absence of copolymer, the reaction rate exhibited a 35-fold decrease as the toehold length of initiator decreased from 8 to 4 nucleotide (nt). In contrast, the reaction rate of CHA in the presence of PLL-*g*-Dex only decreased 40% when the toehold was truncated from 8 nt to 4 nt. The copolymer promoted the hybridization at toehold region, shifting the rate-limiting step to branch migration. Short toeholds can reduce the undesired hybridization between the initiator and bystander DNA strand. Therefore, the combination of short toeholds and use of the copolymer enhanced the robustness of CHA circuit to molecular noise such as the interference from bystander DNA molecule. The function of PLL-*g*-Dex in reducing the impact of toehold length was further demonstrated by analyses of two-input AND logic circuit. Without PLL-*g*-Dex, the truncation of toehold lengths of two inputs or the low salt concentration resulted in the incorrect operation of AND circuit, while the presence of PLL-*g*-Dex ensured the high-speed operation of DNA circuit with various toehold lengths and different salt conditions.

In conclusion, this study demonstrated that use of the cationic copolymer is a simple, versatile and efficient strategy to augment the operation rate and robustness of dynamic DNA circuits. This approach paves the way for more flexible design and broader applications of DNA circuits.

備考 ： 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。
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