Circular DNA nanostructure facilitates autocatalysis by restricted Cas12a activation

Yi Li [a,b,*] , *Biyao Yang* [a,b], *Fei Deng* [a,b], *Ewa M. Goldys* [a,b]

[a] Graduate School of Biomedical Engineering, Faculty of Engineering, University of New South Wales, Sydney 2052, Australia
[b] ARC Centre of Excellence for Nanoscale Biophotonics, University of New South Wales, Sydney 2052, Australia
Presenting and Corresponding Author E-mail Address: yi.li6@unsw.edu.au,

Endonuclease Cas12a, as one key part of the CRISPR/Cas biotechnology, possessed a unique dual enzymatic function which has been widely used for various applications[1]. However, the limits *trans*-cleavage efficiency of a single Cas12a ribonucleoprotein (RNP) holds a major barrier for its further growth as a novel biotechnology toolbox[2]. Unlike any previous studies, we, for the first time, reported the manipulation of Cas12a activity with special DNA nanostructure to establish a novel Autocatalytic CRISPR/Cas12a Amplification Reaction (AutoCAR) system, which is able to break the standard reaction pattern of the Cas12a RNP. In our test, >1,000 Cas12a RNPs can be activated with the presence of a single targeted nucleic acid sequence. We carefully investigated the autocatalysis reaction activity of AutoCAR and also its potential mechanism beneath the changed linear to exponential reaction pattern. In addition, to demonstrate the potential value of AutoCAR in bioanalysis, its autocatalysis reaction can be transferred into a groundbreaking approach to existed CRISPR/Cas12a diagnostics, and realized 1 attomole sensitivity to nucleic acid detections at room temperature without the need for any additional amplifications. Furthermore, 1 attomole RNA detection has also been realized by using modified AutoCAR without the need for reverse transcription. These capabilities have been used to detect ~1 copy/μL DNA/RNA targets from *H. pylori* or SARS-CoV-2 genomes, respectively. The development of AutoCAR served as an exploration to manipulate the Cas enzyme activity patterns, and revealed the under covered potentials of CRISPR/Cas biotechnology for a boarder range of applications, such as biochemistry, bioanalysis and diagnostic applications.

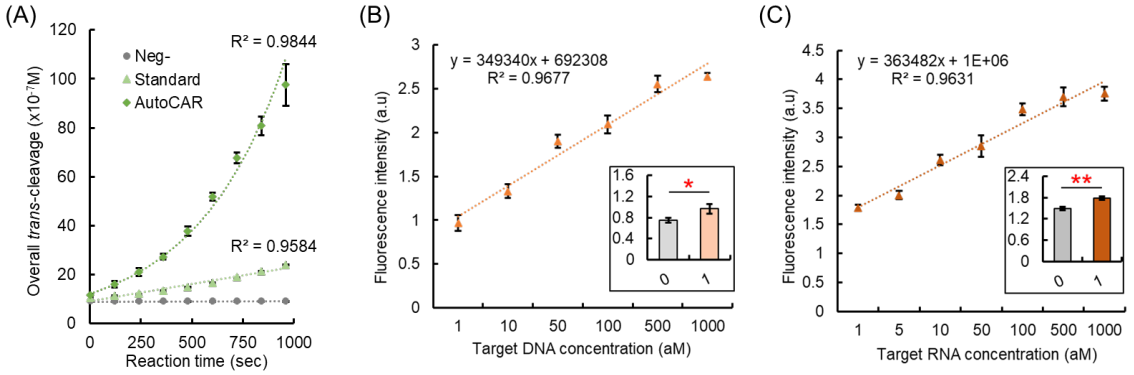

Figure 1: AutoCAR system for ultra-sensitive nucleic acid detection. (A) The fluorescence signal increase pattern for AutoCAR in comparison to a standard Cas12a *trans*-cleavage pattern. Instead of a linear pattern for standard CRISPR/Cas12a *trans*-cleavage reaction, AutoCAR produces a nonlinear signal increase. Differences in *trans*-cleavage reaction kinetics in a standard Cas12 a catalytic system (linear trend, y = 0.014x + 9.1129, goodness of fit R^2=0.9584) and AutoCAR system (super-linear pattern, exponential fit, y = $12.102e^{0.0023x}$, goodness of fit R^2=0.9844). (B) The calibration curve for AutoCAR DNA detection. The system has 3 orders of magnitude linear range with sensitivity of 1 aM for DNA detection. (C) The AutoCAR calibration curve for RNA detection. The system investigated here shows 3 orders of magnitude linear range with maximum sensitivity of 1 aM for RNA detection without reverse transcription.

References

[1] Li, Y., *et al*. *Trends Biotechnology* **2019**, *37* (7), 730-743.
[2] Ramachandran, A., *et al*. *Analytical Chemistry* **2021**, *93* (20), 7456-7464.