CRISPR/Cas12a シグナル増幅機能を活用した折り紙型免疫測定デバイス

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CRISPR/Cas12a-Based Signal-Amplified Origami Device for Immunoassay (*Graduate School of Science and Technology, Keio University*) ○Hikaru Suzuki, Guodong Tong, Yuki Hiruta, Daniel Citterio

Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas12a, which represent one type of endonucleases, exhibit a collateral activity, which randomly cleaves non-targeted single stranded DNA (ssDNA) in their proximity when specifically recognizing a target double strand DNA (dsDNA). Biosensing approaches based on this so-called *trans*-cleavage activity have gained strong focus as a method to detect nucleic acids. However, few CRISPR-based biosensors have been developed for non-nucleic acid detection. We present here a simple, rapid, and highly sensitive detection method for non-nucleic acid targets by integrating the CRISPR/Cas12a system and an enzyme-linked immuno-sorbent assay (ELISA). An origami paper-based device has been designed, and the detection of targets on the paper substrate is based on the measurement of the fluorescence signal caused by Cas12a cleaving a probe ssDNA, which has been labeled by a fluorescent dye and a quencher at both ends, respectively.

It was confirmed that the fluorescence signal due to *trans*-cleavage was observed on the paper substrate only when Human IgG, which was used as a model target, was present. Subsequently, parameters such as the reagent concentration, volume and reaction time were optimized, and we successfully obtained concentration response for Human IgG.
Keywords : CRISPR/Cas12a; DNA; Immunoassay; ELISA; Paper-based analytical device

CRISPR/Cas12a は、核酸分解酵素の一つであり、標的の二本鎖 DNA を特異的に認識すると同時に、周囲の非標的一本鎖 DNA を無作為に切断する（トランス切断）という特徴をもつ。このトランス切断を利用したバイオセンシング技術は、核酸を高感度に検出する方法として注目を集めているが、非核酸分子の検出への適用例は少ない[1]。そこで本研究では、CRISPR/Cas12a システムと ELISA を組み合わせることで、非核酸分子を迅速、簡便、高感度に検出する方法を開発した。折り紙型の紙基板デバイスをデザインし（Fig. 1）、紙基板上において Cas12a が一本鎖 DNA（両端に蛍光色素とクエンチャーを標識したもの）を切断することで生じた蛍光を測定する。

実際に折り紙型のデバイスを使用し、紙基板上でトランス切断による蛍光が観測できることを確認した。その後、試薬濃度・塗布量、反応時間などのパラメータを最適化し、モデルターゲットであるヒト IgG の濃度応答を得ることに成功した。

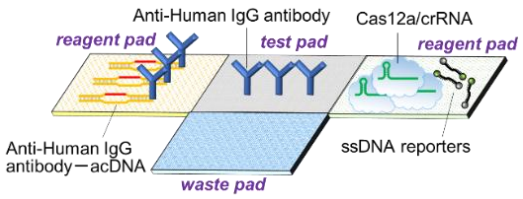

Fig. 1 Device design

1) Xiong, Y. *et al.*, *J. Am. Chem. Soc.* **2020**, *142* (1), 207–213.