Bringing autocatalysis to CRISPR sensors: could PCR tests become redundant?

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Programmable nucleases like Cas12a present unprecedented possibilities for nucleic acid biosensors. These sensors provide sequence specificity, eliminate the need for sample preparation or washing steps, and offer versatility and universality. However, their sensitivity is limited approximately to a picomolar range which is insufficient for clinical applications.

We developed a new CRISPR sensing technology offering tests which is as sensitive as the lab-based PCR tests but offer rapid, on-the-spot gene-based detection[1]. The key component are small DNA nano-circles (~ 2nm size) containing a short sequence of the target DNA. They are cpmbines with Cas12a proteins programmed to cut DNA of the nano-circles – but only when activated by DNA from the targeted sequence. The interaction of a suitably programmed CRISPR/Cas protein with the gene target we are trying to detect causes the DNA nano-circles to break up, linearise and become 'fake targets'. This closes the autocatalytic loop producing a molecular chain reaction. This then creates catalytic products which are easy to detect even with lateral flow devices. Autocatalysis is also sustained with DNA nanostructures modified with fluorophore-quencher pairs achieving 1 aM level (<1 copy/uL) DNA detection (10^6 times improvement), without additional amplification, within 15 mins, at room temperature. The detection range is tuneable, spanning 3 to 11 orders of magnitude. We demonstrate 1 aM level detection of SNP mutations in circulating tumour DNA from blood plasma, genomic DNA (*H. Pylori*) and RNA (SARS-CoV-2) without reverse transcription as well as colorimetric lateral flow tests of cancer mutations with ~100 aM sensitivity.

Reference

[1]Deng, F., Li, Y., Yang, B. *et al.* Topological barrier to Cas12a activation by circular DNA nanostructures facilitates autocatalysis and transforms DNA/RNA sensing. *Nat Commun* **15**, 1818 (2024). https://doi.org/10.1038/s41467-024-46001-8