Topological barrier to Cas12a activation by circular DNA nanostructures facilitates autocatalysis and transforms DNA/RNA sensing

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Control of CRISPR/Cas12a trans-cleavage is crucial for biosensor development. Here, we show that small circular DNA nanostructures which partially match guide RNA sequences only minimally activate Cas12a ribonucleoproteins. However, linearizing these structures restores activation. Building on this finding, an Autocatalytic Cas12a Circular DNA Amplification Reaction (AutoCAR) system is established which allows a single nucleic acid target to activate multiple ribonucleoproteins, and greatly increases the achievable reporter cleavage rates per target. A rate equation-based model explains the observed near-exponential rate trends. 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.