Simple and rapid detection of protein/small molecule interaction based on CRISPR/Cas12a collateral cleavage activity

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To realize the full potential of the CRISPR/Cas system and expand its applicability up to the detection of molecular interactions, we developed a novel method to identify protein/small molecule interactions by utilizing the CRISPR/Cas12a collateral cleavage activity. This technique employs a single-stranded activator DNA modified with a specific small molecule, which would switch on the CRISPR/Cas12a collateral cleavage activity upon binding to crRNA. When the target protein binds to the small molecule on the activator DNA, the bound protein sterically hinders the access of the activator DNA to crRNA, thereby promoting less collateral cleavage activity of CRISPR/Cas12a. As a consequence, fewer reporter probes nearby are cleaved to produce accordingly reduced fluorescence signals in response to target protein. Based on this unique design principle, streptavidin/biotin and anti-digoxigenin/digoxigenin were successfully determined down to 0.03 nM and 0.09 nM, respectively, with a fast and simple detection workflow (11 min). The practical applicability of this method was also verified by reliably detecting target streptavidin spiked in heterogeneous human serum.